



XylPhi-PD[®]

Bactericide for use in grapevines.

For *biologically based* reduction of Pierce's Disease (PD) in grapevines.

Fully registered and available for commercial sale in certain US states.



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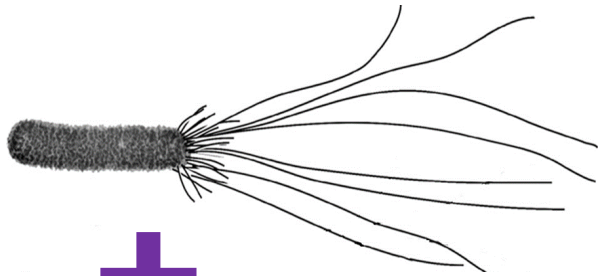
Today's Talk

- Pierce's Disease ("PD") Overview
- Bacteriophage Overview – XylPhi-PD[®]
- Xyleject – how to apply
- Efficacy of XylPhi-PD[®]

Biology of Pierce's Disease

1. *Xff* colonizes the gut of sharpshooters.

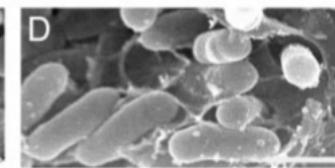
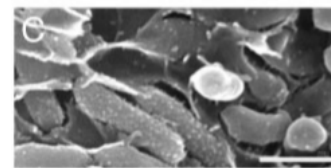
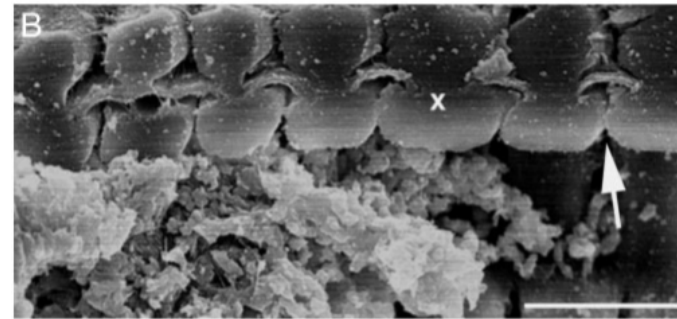
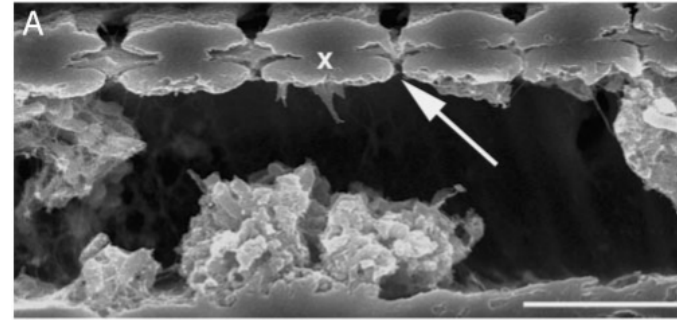
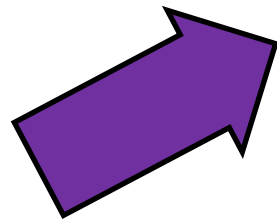
Xylella fastidiosa (*Xff*)



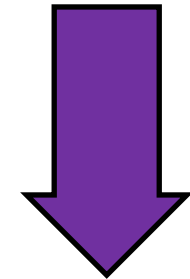
Glassy-winged sharpshooter



Blue-green sharpshooter



2. *Xff* forms biofilms in the xylem tissue of grapevines.



3. *Early symptoms of Pierce's Disease mimic water stress.*



Management Practices for Pierce's Disease

Physical Control

- Vineyard inspections and testing
- Pruning-out diseased shoots
- Rogueing entire diseased vines
- Replanting, less susceptible varieties

Vector Control

- Trapping and monitoring
- Insecticide treatments
- Inspection and certification of bulk grape shipments
- Insect parasitoids

Additional Measures

- Situating vineyards away from pathogen and vector sources
- Removal of alternative host plants
- Transgenic & PD-resistant vines**
- Bacterial endophytes-competitive exclusion, peptides**
- Bacteriophages**

Classic approaches

University of California IPM publishes management guidelines and updates on R&D related to classical approaches.

Recent or potential approaches

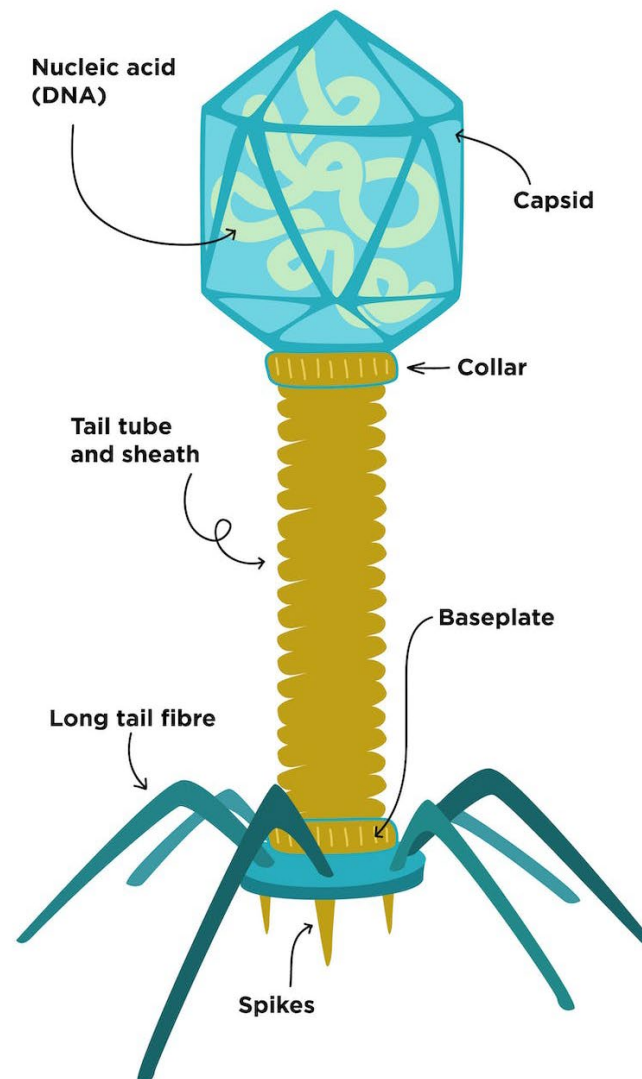
For recent approaches, XylPhi-PDTM is the only EPA-approved treatment. PD-resistant vines are available, but these vines are still capable of harboring *Xff*.¹

1. UC Davis Foundation Plant Services

The hero: Bacteriophage or 'Phage'

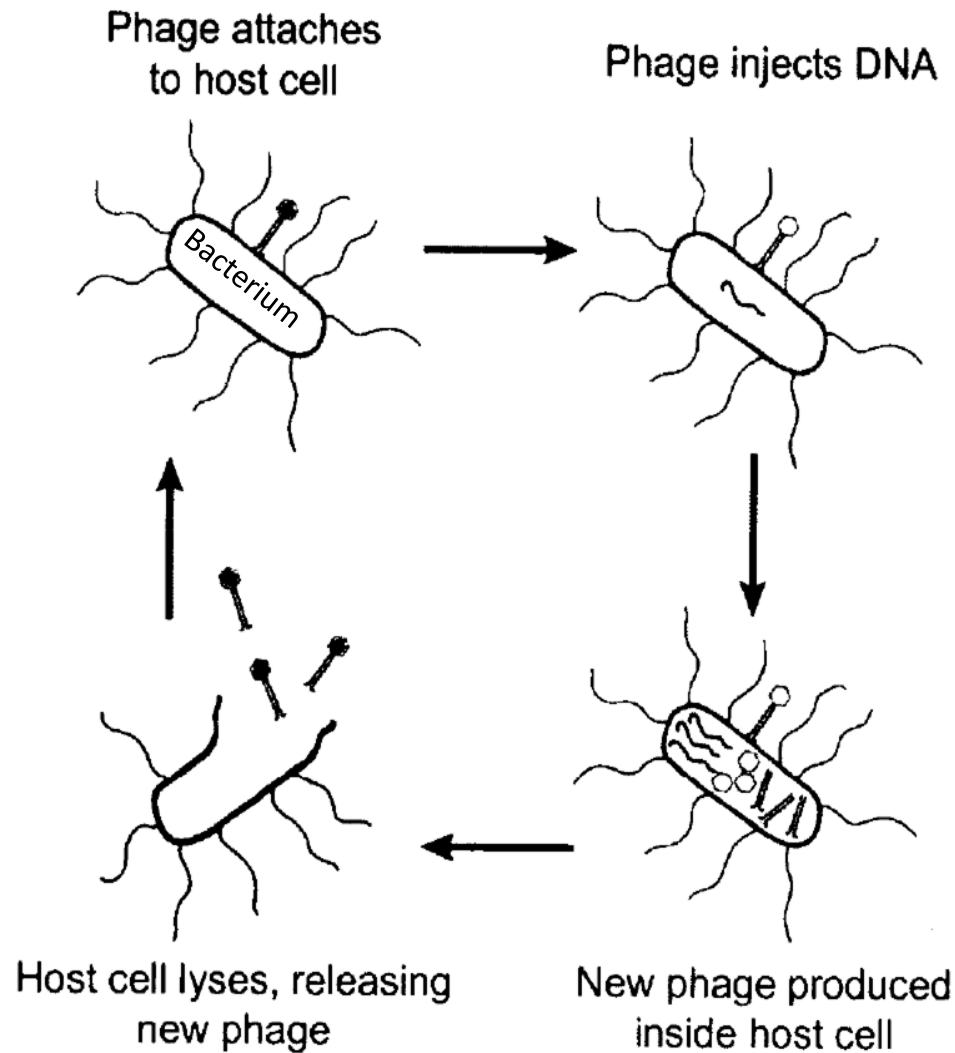
A very selective virus that infects and replicates in bacteria

BACTERIOPHAGE



Bacteriophage: An Old Approach to a Current Problem

Bacteriophage Cycle Overview



Bacteriophage (phage) were first discovered over 100 years ago.

Phages are *bacterium-specific* and harmless to plants, people, and beneficial microflora.

The use of phage as biocontrol agents offers a *novel alternative approach* for the control of Pierce's Disease.

Analogous approaches are being pursued as an alternative to antibiotics for control of human pathogens.

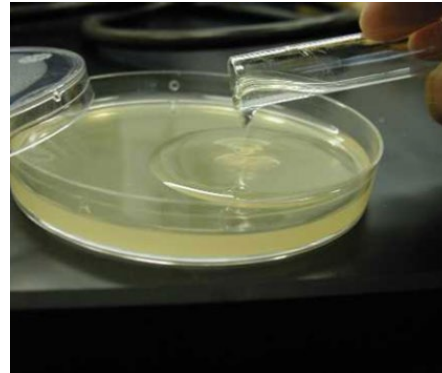
Texas A&M Discovery Process

The first lytic phages to target *X. fastidiosa* subsp *fastidiosa*

1. Collect site-specific environmental samples (e.g., grapevines, weeds, water)



2. Assay samples for phage



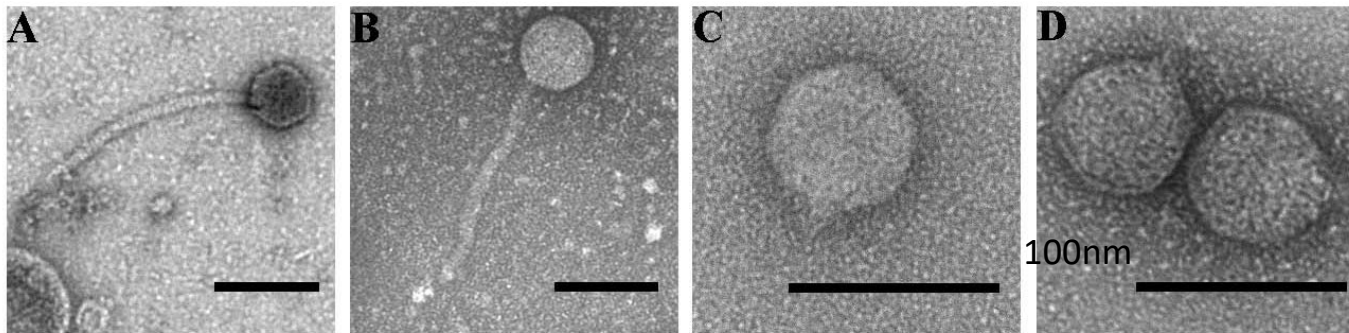
3. Isolate phage active against local *Xff* strains



4. Sequence phages, confirm lack of toxins, & determine lytic lifecycle



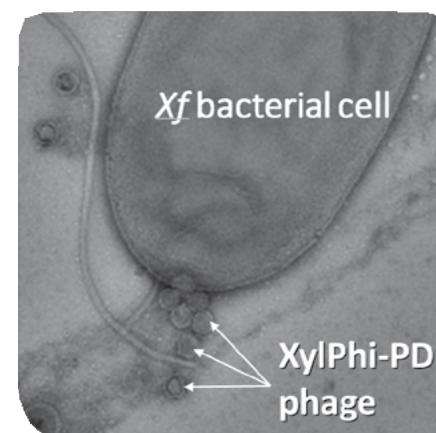
Bacteriophage Library



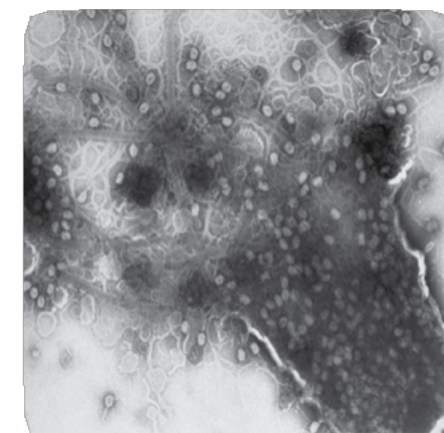
How it works



- A cocktail of viral **bacteriophages** (phages) that enter, attack, and kill *Xylella fastidiosa* (*Xf*) bacteria, the cause of PD.
- Uses the selective biological activity of phages to destroy targeted bacteria in treated grapevines.
- Apply as a **treatment** when early stage disease symptoms appear, or as a **preventative** to protect growing vines.



Viral bacteriophage particles of XylPhi-PD[®] precisely targeting a bacterial host.

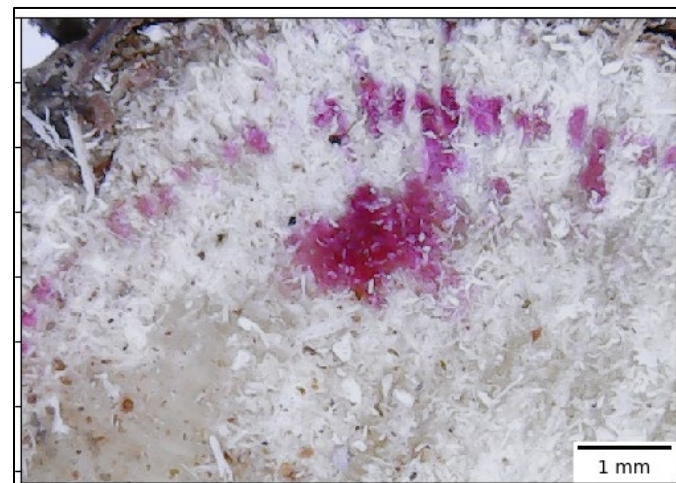


Death and rupture of a bacterial cell, releasing newly created phage particles to seek and destroy more *Xf* cells.

How to apply

- XylPhi-PD[®] is applied by *injection* into the vascular system (xylem) of grapevines.
- The Pulse Xyleject[™] pressurized injection device (from Pulse Biotech)
- Training is available for vineyard staff.

Dye injection demonstrates movement up to a meter within minutes of injection



Texas A&M 2015 Greenhouse Field Trial — Preventive Treatment

Prophylactic or
buffer
treatment



Week 0



Xff
inoculation



Week 3



Visual PD
assessment



Week 0

Week 2

Week 4

Week 6

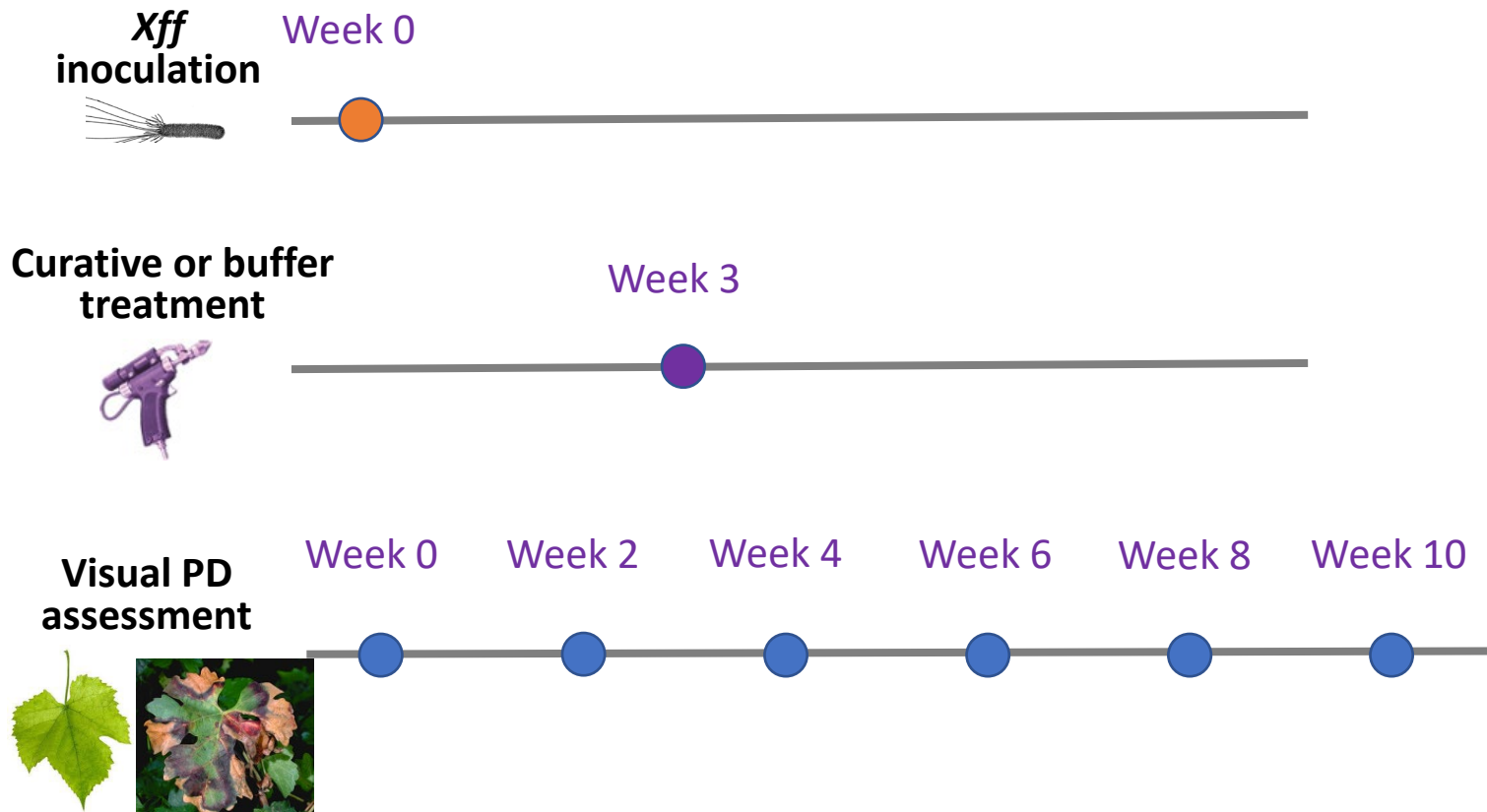
Week 8

Week 10



- Test treatment of Pierce's Disease with application of XylPhi-PD *prior to* *Xff* introduction
- Cabernet Sauvignon
- 1103 Paulson rootstock

Texas A&M 2015 Greenhouse Field Trial — Curative Treatment



- Test treatment of Pierce's Disease with application of XylPhi-PD *after* Xff introduction
- Cabernet Sauvignon
- 1103 Paulson rootstock

Texas A&M 2015 Greenhouse Trial — Visual Assessment Results

Phage only



No phytotoxicity

Xff inoculation



Chlorosis, stunting,
and leaf drop

Xff inoculation then phage

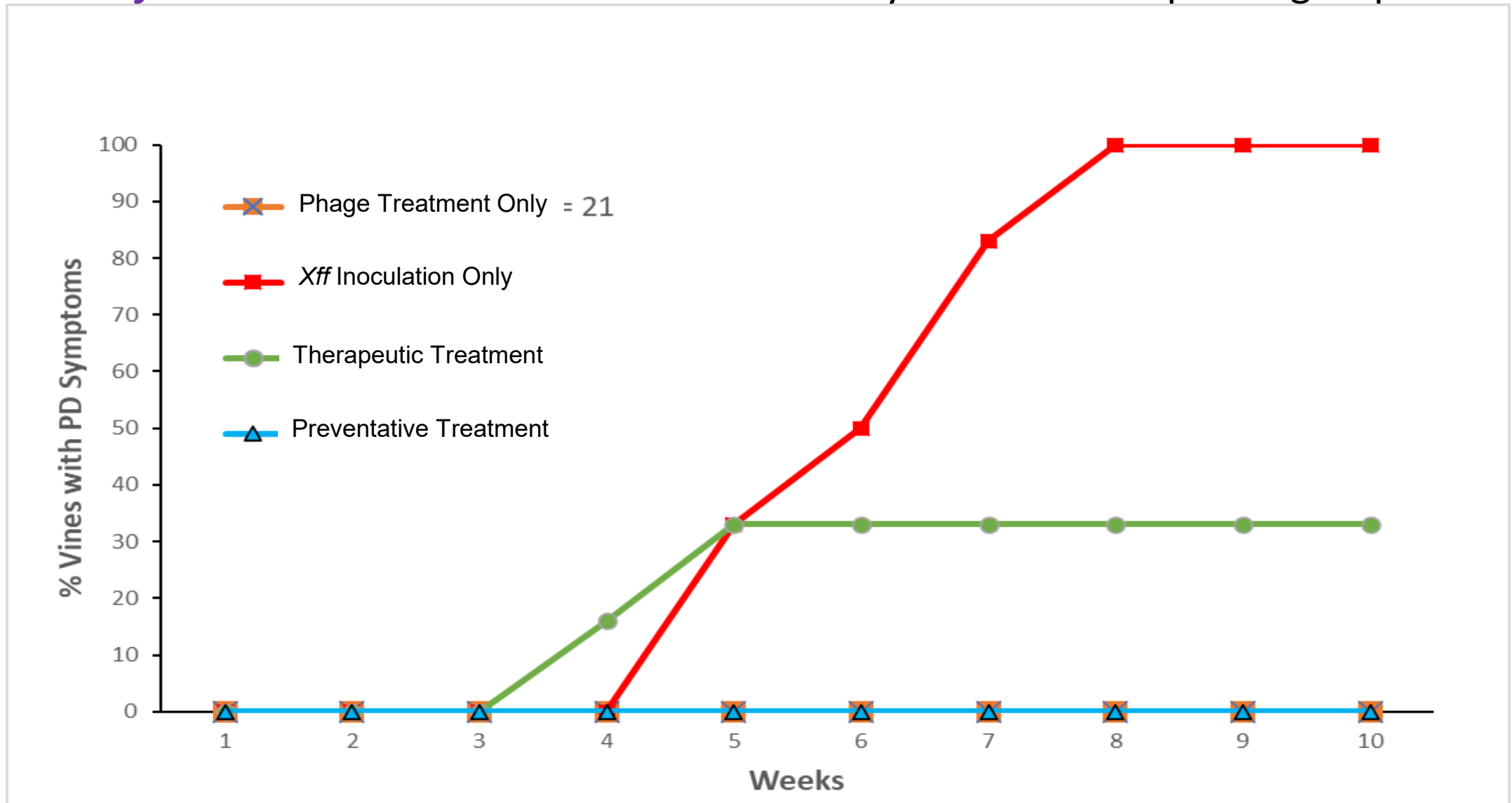


Phage treatment 3 weeks after
Xff inoculation

Phage-treated vines appear healthier 10 weeks post-inoculation

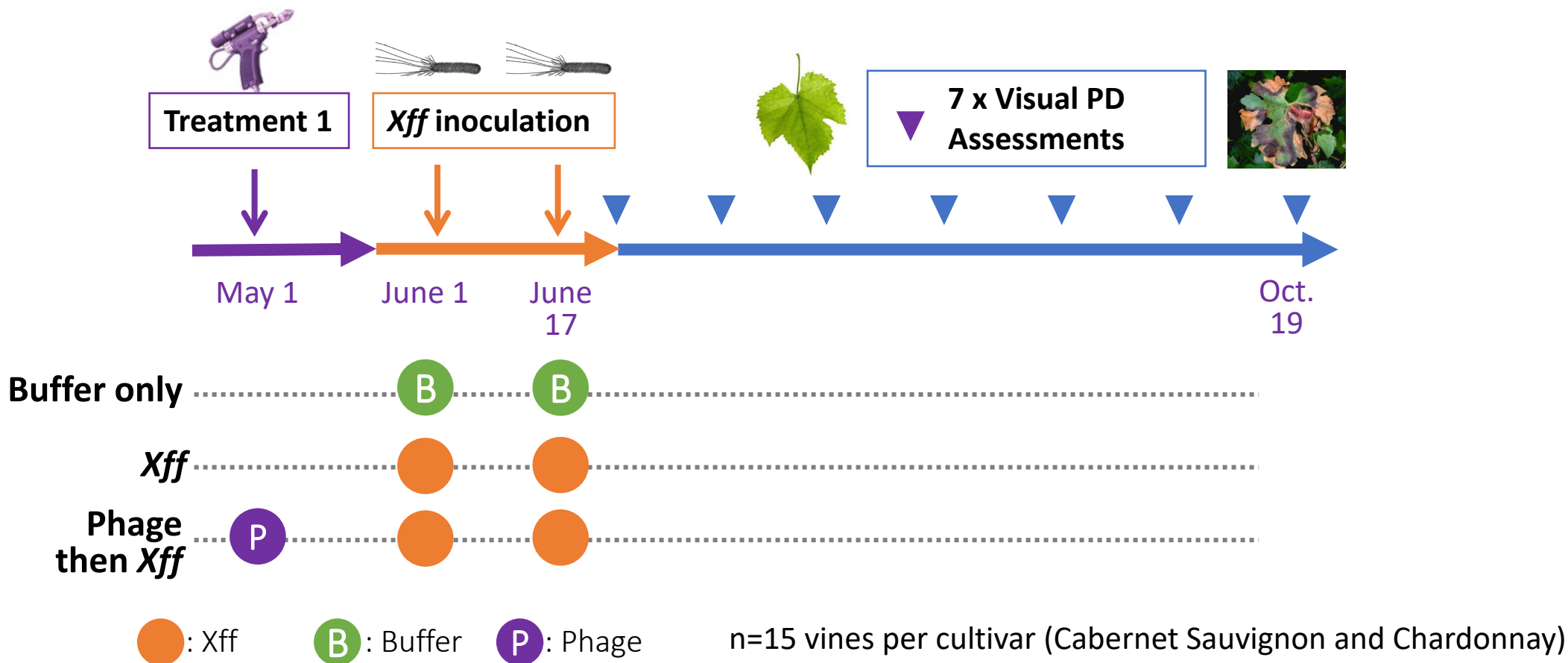
Texas A&M 2015 Greenhouse Trial — Preventive and curative efficacy with XylPhi-PD[®]

- *No cases* of PD were detected in the XylPhi-PD[®] preventative group
- *66% fewer cases* of PD were detected in the XylPhi-PD[®] therapeutic group



California 2017 Field Trial — Preventive Treatment

Test prevention of Pierce's Disease with application of XylPhi-PD prior to *Xff* introduction

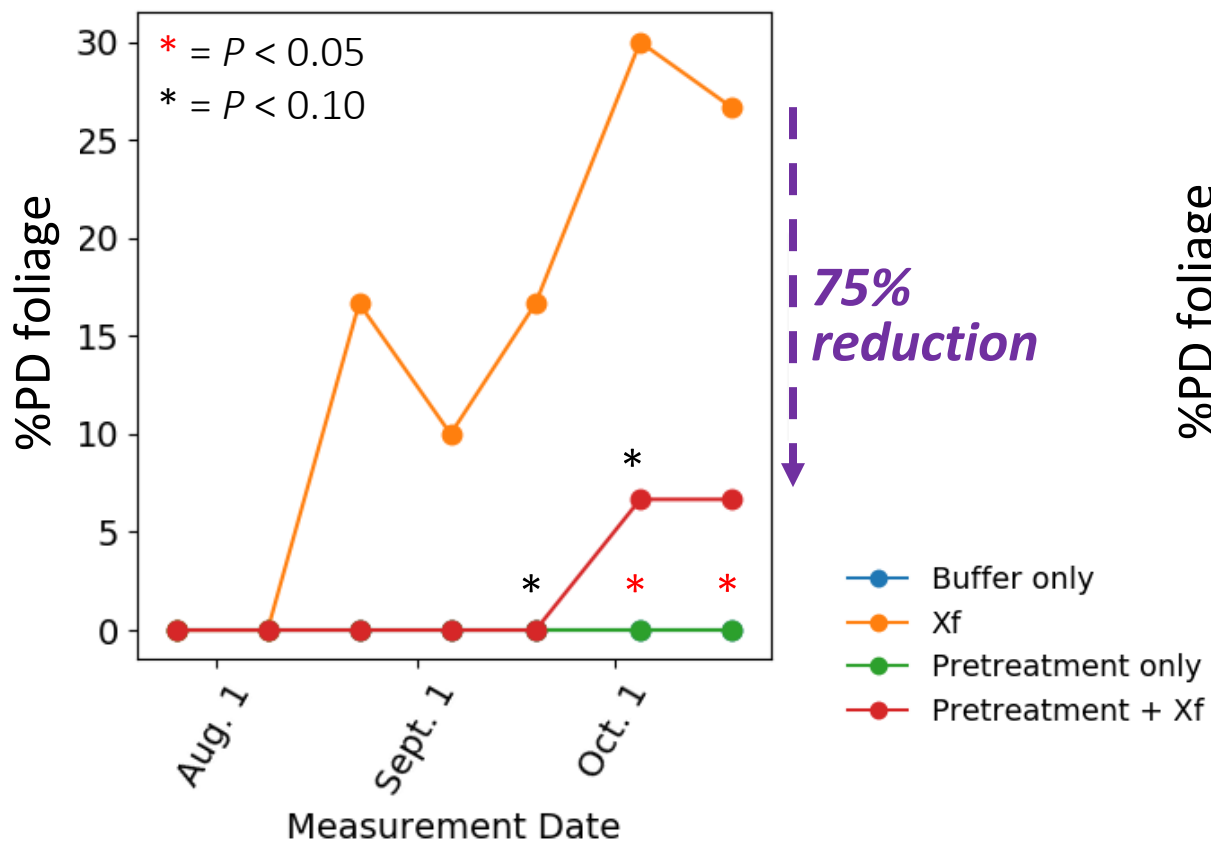


Treatment group	<i>Xff</i> inoculation	# Phage inoculations	# Cabernet Sauvignon vines	# Chardonnay vines
Buffer treatment	No	0	15	15
<i>Xff</i> inoculation only	Yes	0	30	30
Phage treatment then <i>Xff</i> inoculation	Yes	1	15	15

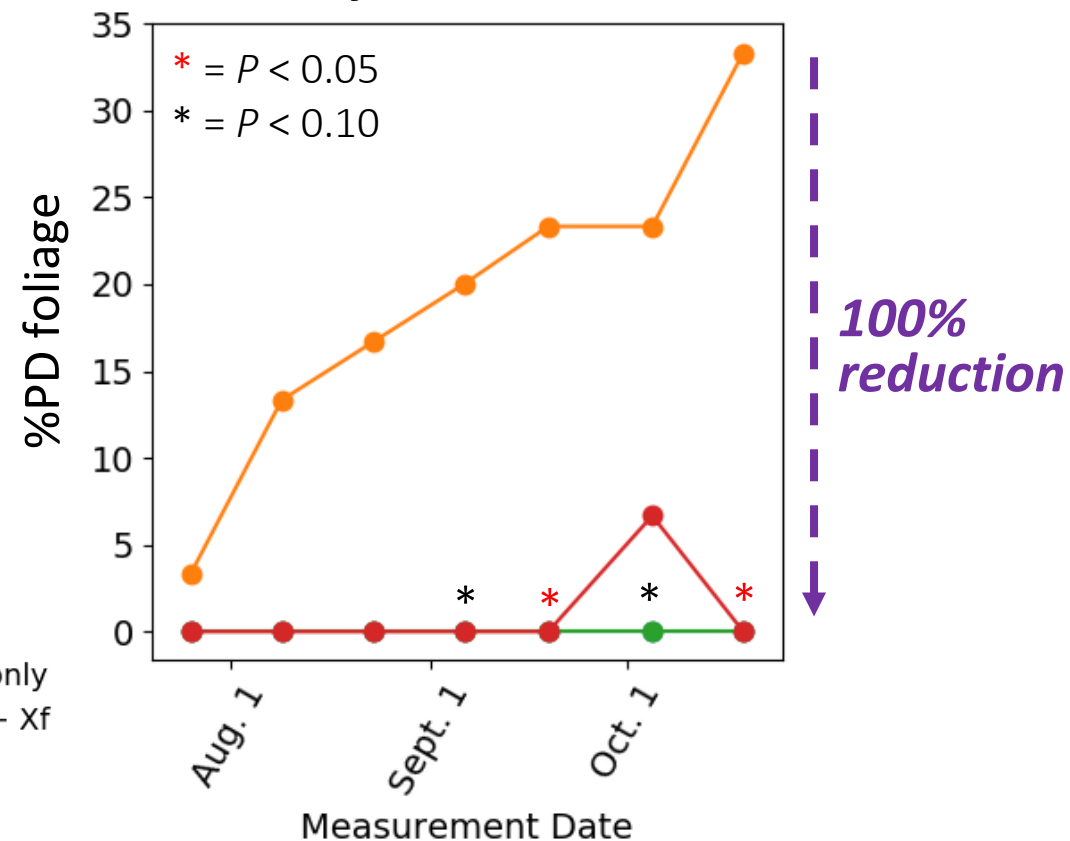
California 2017 Field Trial Results — Preventive Treatment

- Efficacy assessed at 20 weeks after *Xff* inoculation in varieties with different PD susceptibility.
- Incidence of PD symptoms significantly reduced by **75%** in Cabernet Sauvignon vines and **100%** in Chardonnay vines.

Cabernet Sauvignon

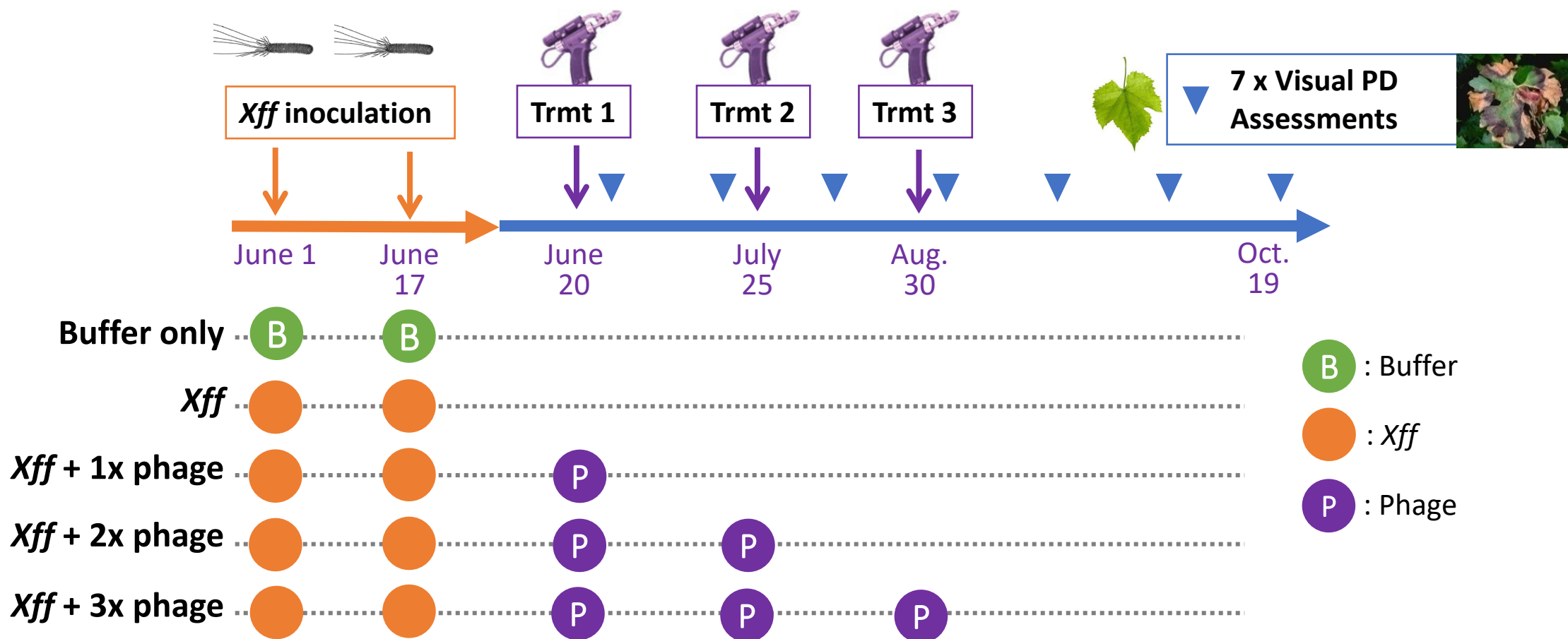


Chardonnay



California 2017 Field Trial — Curative Treatment

Test treatment of Pierce's Disease with application of XylPhi-PD after *Xff* introduction

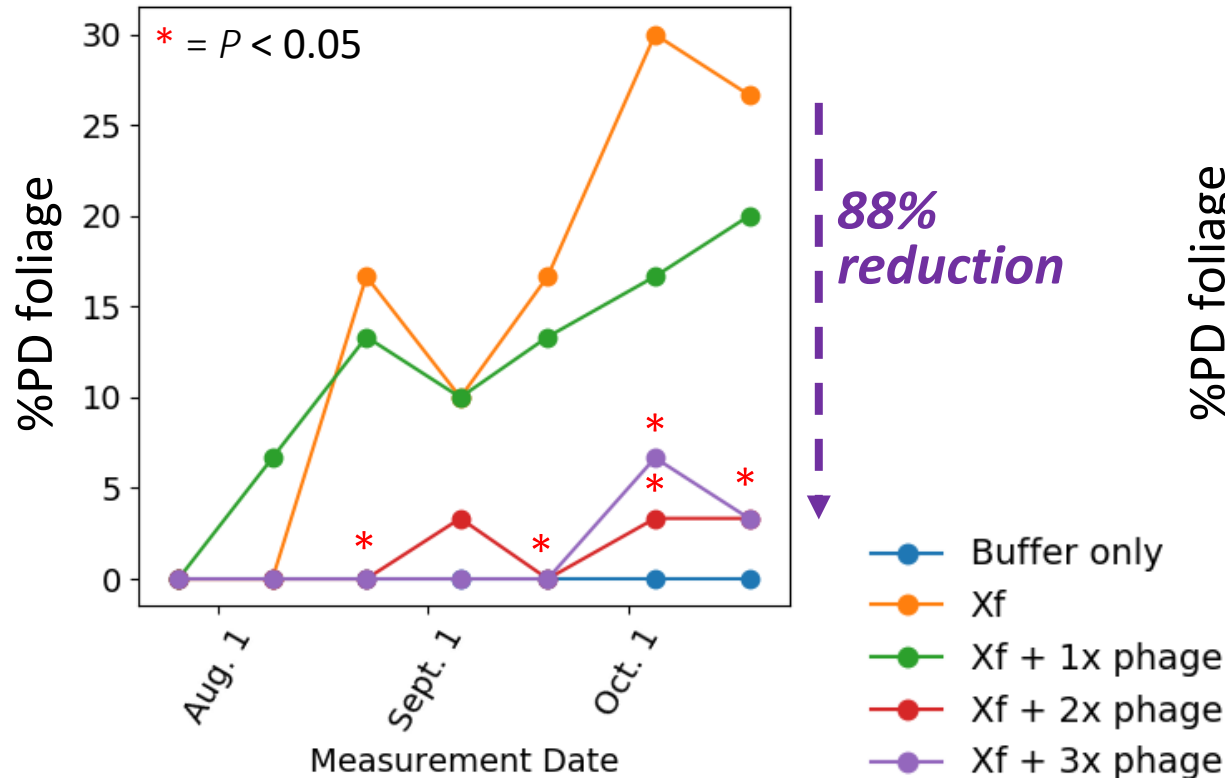


Treatment group	<i>Xff</i> inoculation	# Phage inoculations	# Cabernet Sauvignon vines	# Chardonnay vines
Buffer only	No	0	30	30
<i>Xff</i> inoculation	Yes	0	30	30
1x phage	Yes	1	30	30
2x phage	Yes	2	30	30
3x phage	Yes	3	30	30

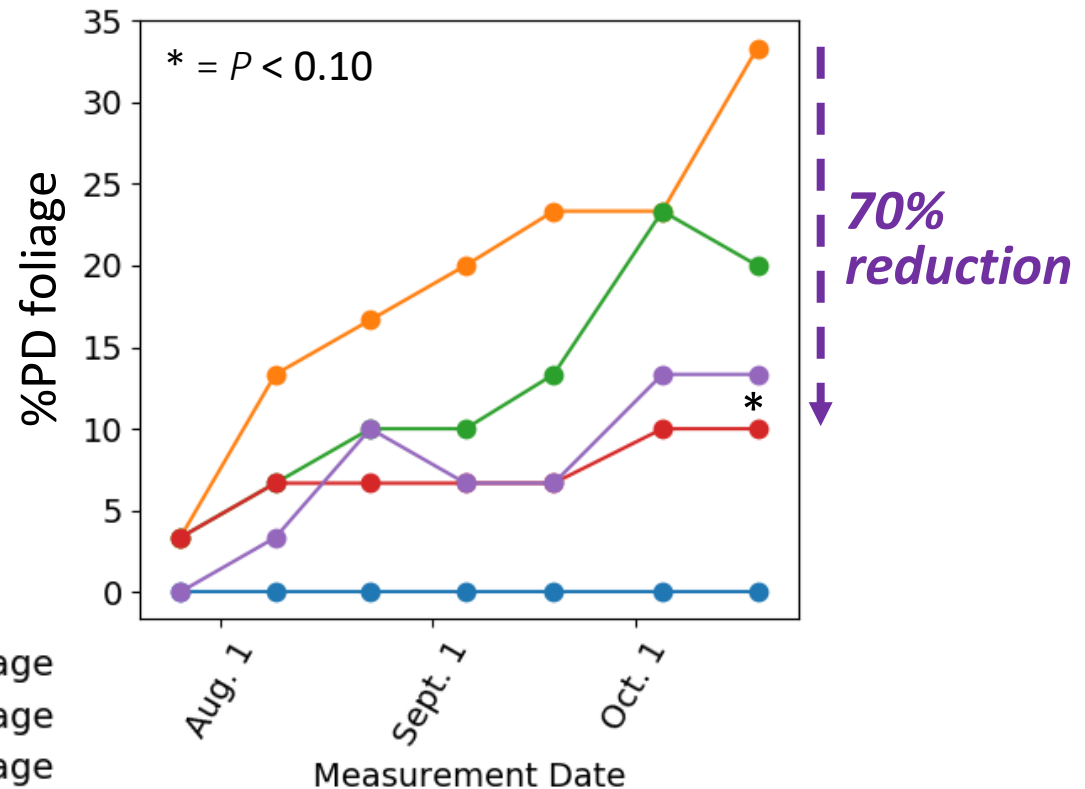
California 2017 Field Trial Results — Curative Treatment

- Efficacy assessed at 20 weeks after *Xff* inoculation in varieties with different PD susceptibility.
- The incidence of PD symptoms was significantly reduced by **88%** in Cabernet Sauvignon vines and **70%** in Chardonnay vines.
- Multiple phage treatments were significantly better than a single phage treatment for control of the incidence of PD-symptomatic vines.

Cabernet Sauvignon

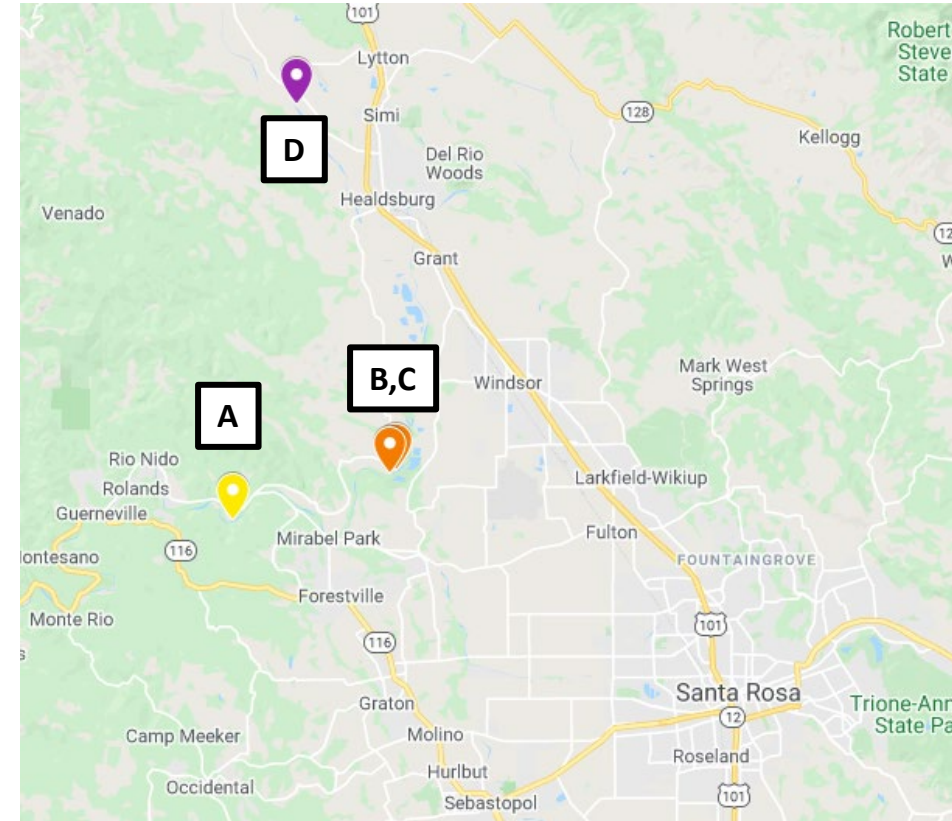


Chardonnay



CA 2019-2021 Wilbur-Ellis Field Trial — Design

- Sites with historical high PD pressure chosen in Napa/Sonoma counties
 - 4 sites used
 - 2 sites (B,C) were separate blocks in the same vineyard
- Trial locations near or adjacent to a riparian edge
- All sites commercial vineyards
- Each trial site divided into 4 treatment groups



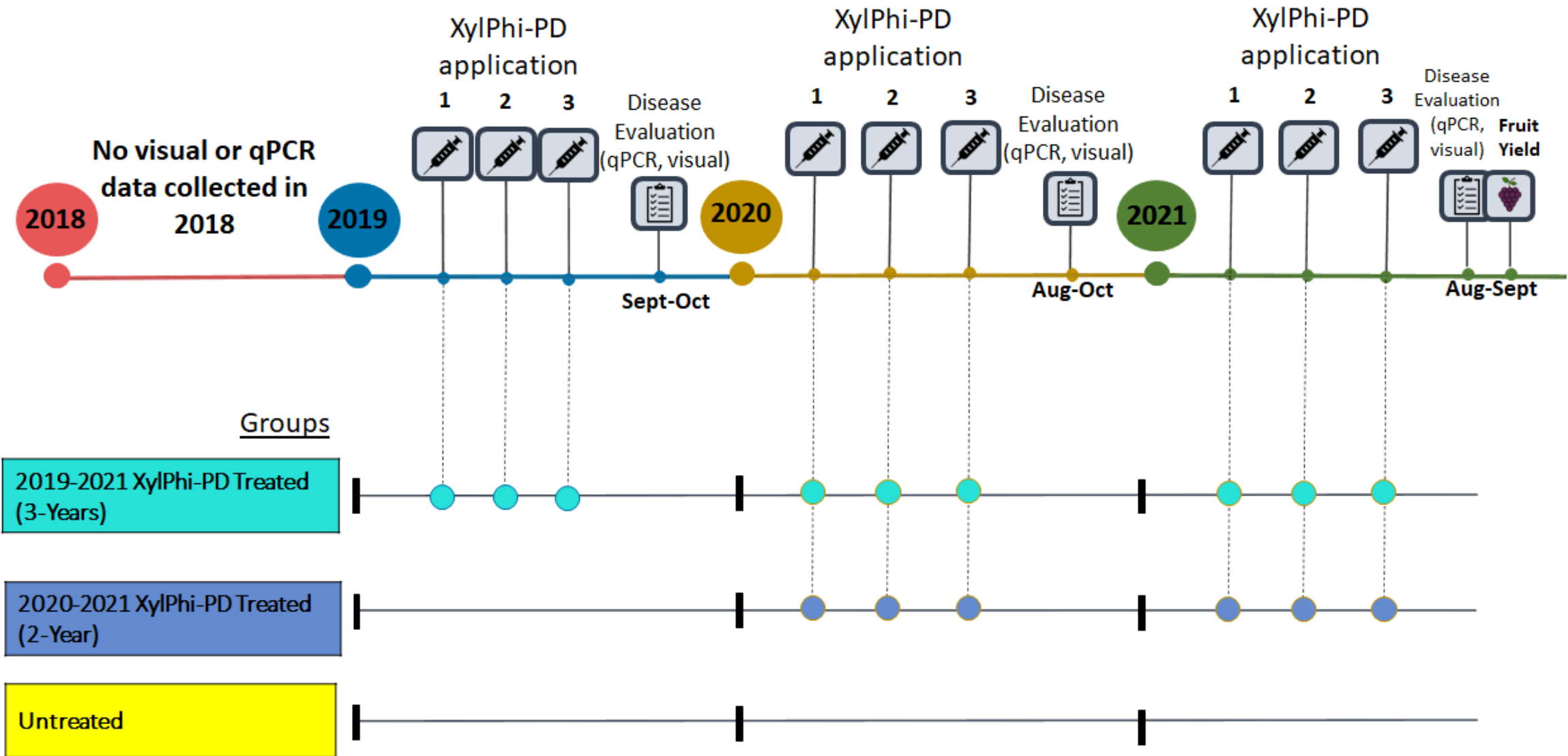
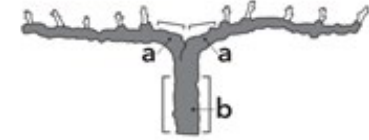
Site	Location	Cultivar
A	Russian River	Chardonnay
B	Russian River	Pinot noir
C	Russian River	Chardonnay
D	Dry Creek	Chardonnay



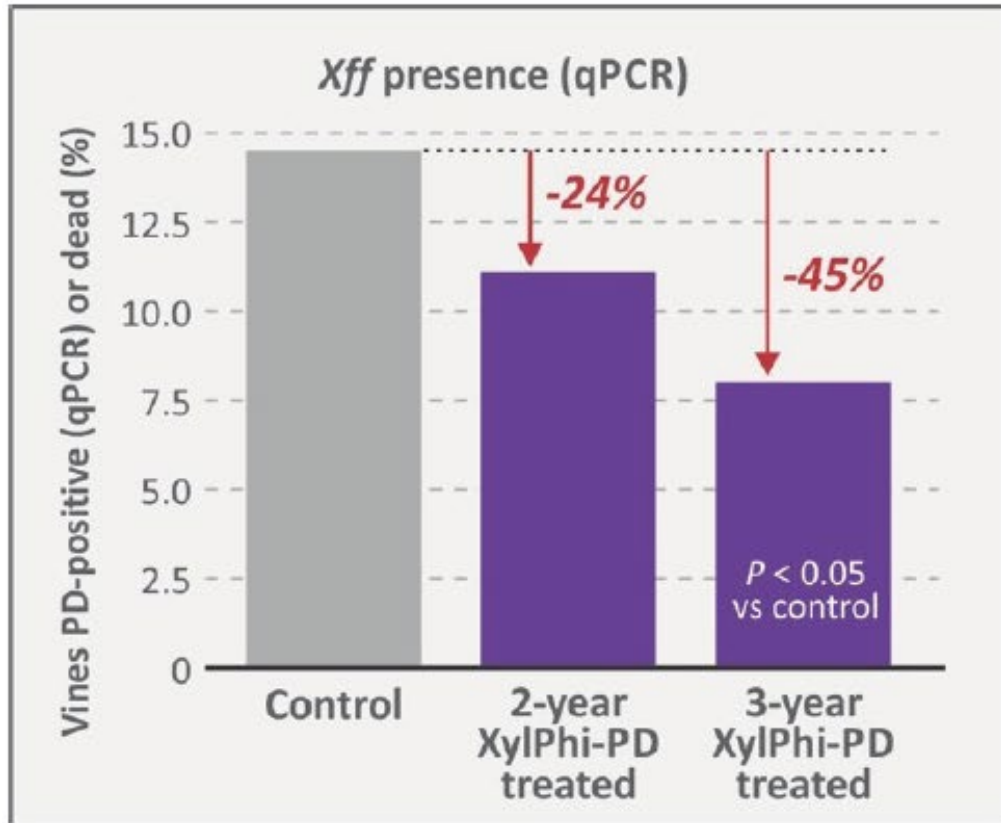
- 150 vines per site (600 vines total)
- All vines enrolled in the study are 8+ years old

Wilbur Ellis 4-Site Study is in its 3rd year

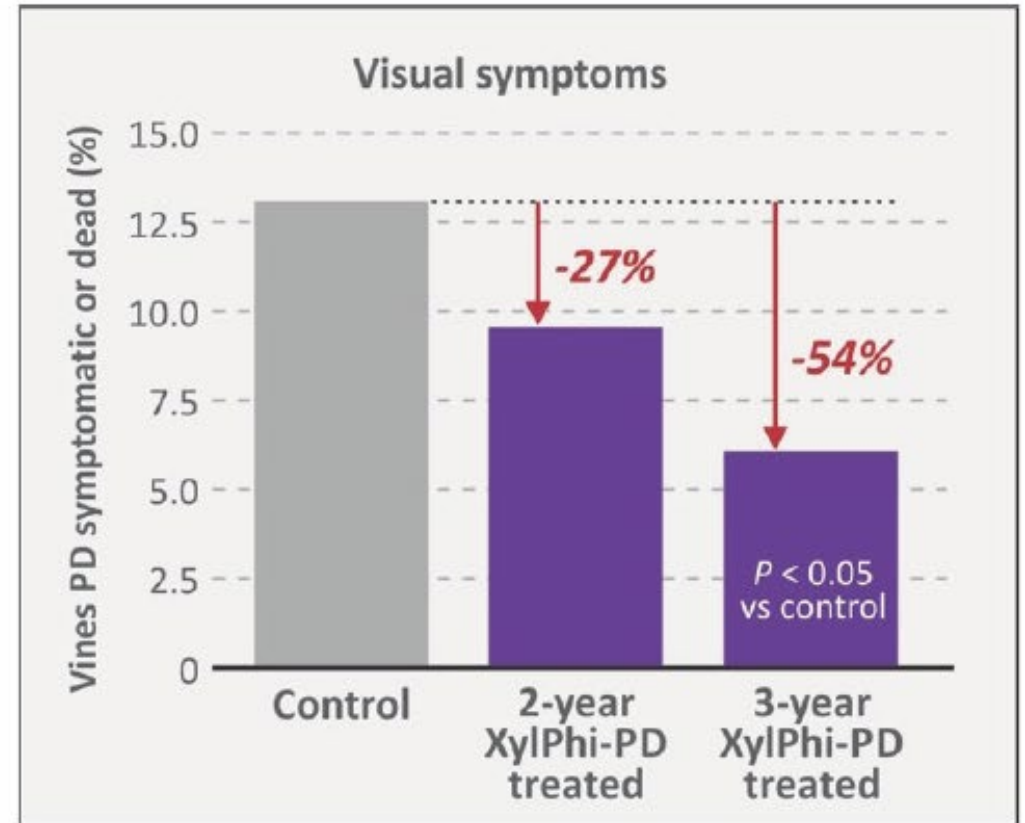
Treatment: 4 injections (80 μ L each) of XylPhi-PD per vine



Wilbur Ellis 4-Site 3-year Efficacy

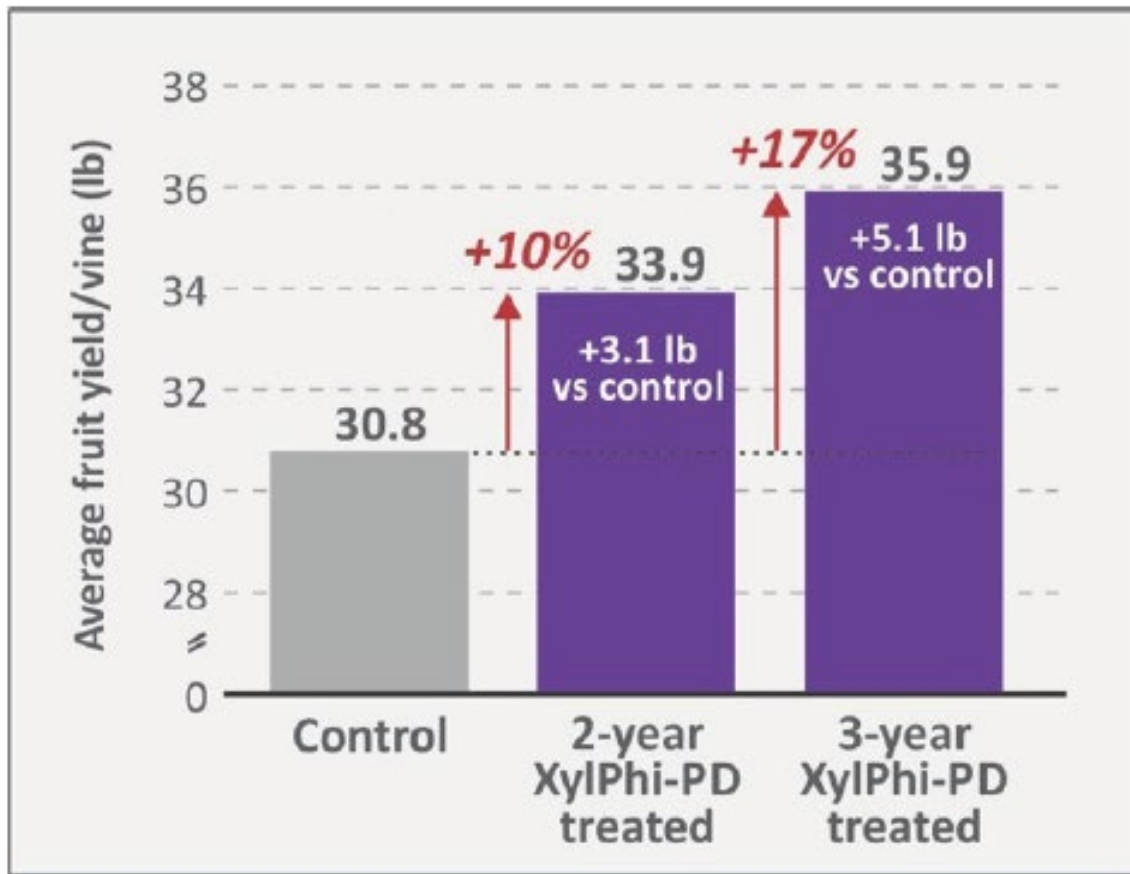


Incidence of *Xff* positivity fell 24% and 45%, respectively, for vines receiving two or three years of treatment compared to untreated controls.



Number of vines displaying PD symptoms also fell 27% and 54%

Wilbur-Ellis 4-Site Study: Yield



- Fruit yield per vine was measured on all 150 vines (50 vines/treatment group) at Site D in Fall 2021
- 10% and 17% more fruit in the 2-year treated and 3-year treated vines, respectively

CA 2019-2020 Wilbur-Ellis Field Trial —

Conclusions

- In the 4-sites with ongoing, high PD pressure in 2020, vines treated with XylPhi-PD recovered much more often than untreated vines, both in visual symptomology and presence of *Xff* as measured by qPCR.

PD visual diagnosis results:

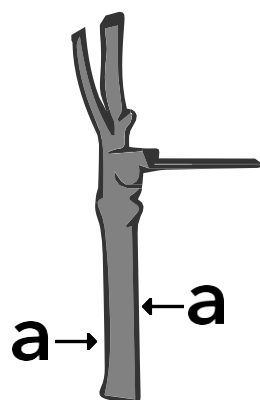
- **60% fewer vines** in the ‘2019 & 2020 XylPhi-PD Treated’ group displayed PD symptoms
- **35% fewer vines** in the ‘2020 XylPhi-PD Treated’ group displayed PD symptoms
- 25% fewer vines in the ‘Untreated’ group displayed PD symptoms
 Related to the lower PD pressure seen in 2020 through both low sharpshooter counts and some recovery from PD in the untreated group

qPCR detection of *Xff* results:

- **57% drop** in measurable *Xff* in the ‘2019 & 2020 XylPhi-PD Treated’ group
- 14% fewer vines in the ‘Untreated’ group had measurable *Xff*

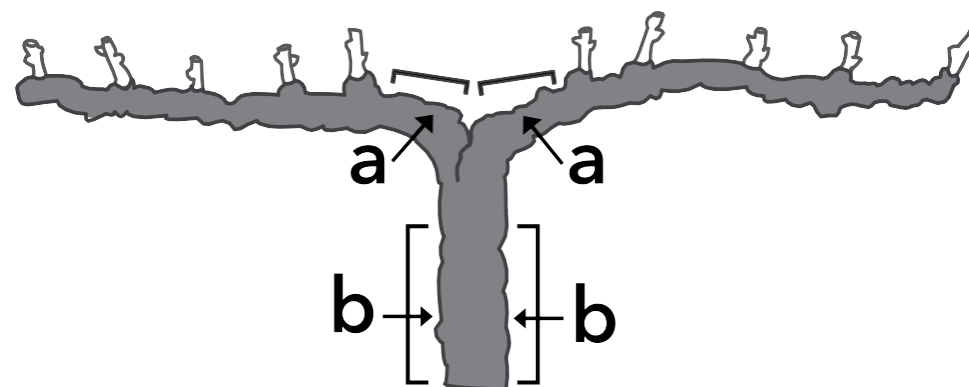
Where to inject the vine

□ *Replants and young vines*



- 2 injections in opposite sides of the trunk (a)

□ *Mature vines*

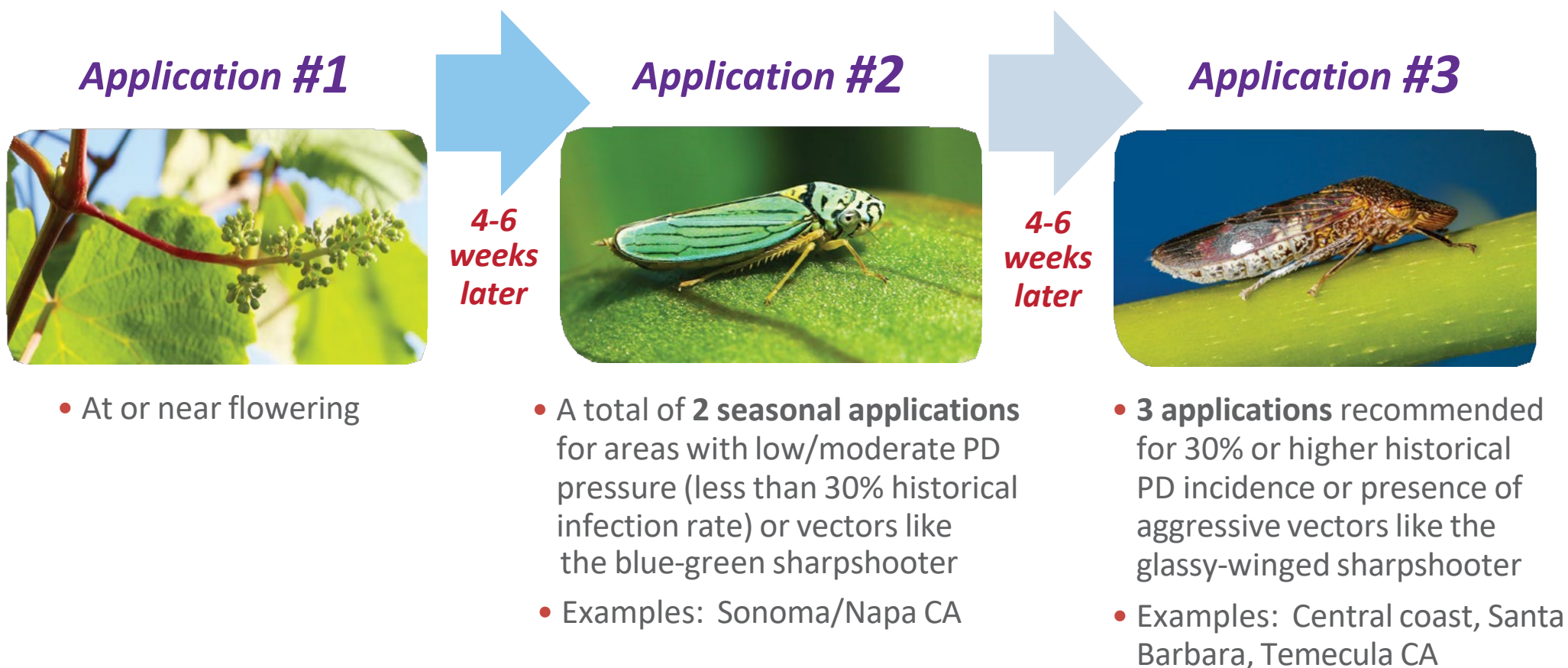


- 1 injection in each cordon (a)
- 2 injections in the trunk (b)

Timing: Begin seasonal applications at flowering, then apply on a 4-6 week interval up to 3 times / season depending on pressure

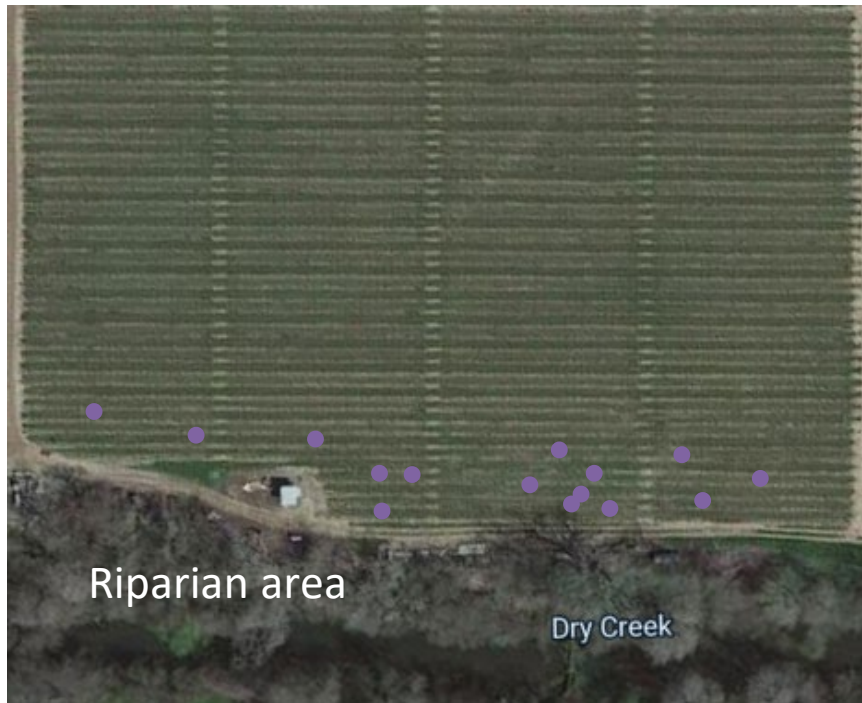
When to apply

Make 2 or 3 applications of XylPhi-PD[®] per season, at 4- to 6-week intervals.



Targeted Treatment Program — Buffer Zone

Vines with Pierce's Disease symptoms



XylPhi-PD® suggested treatment area



In areas with >2% of vines displaying PD symptoms, especially when BGSS are the insect vector

Target treatment to areas with high PD activity, to create a buffer zone.

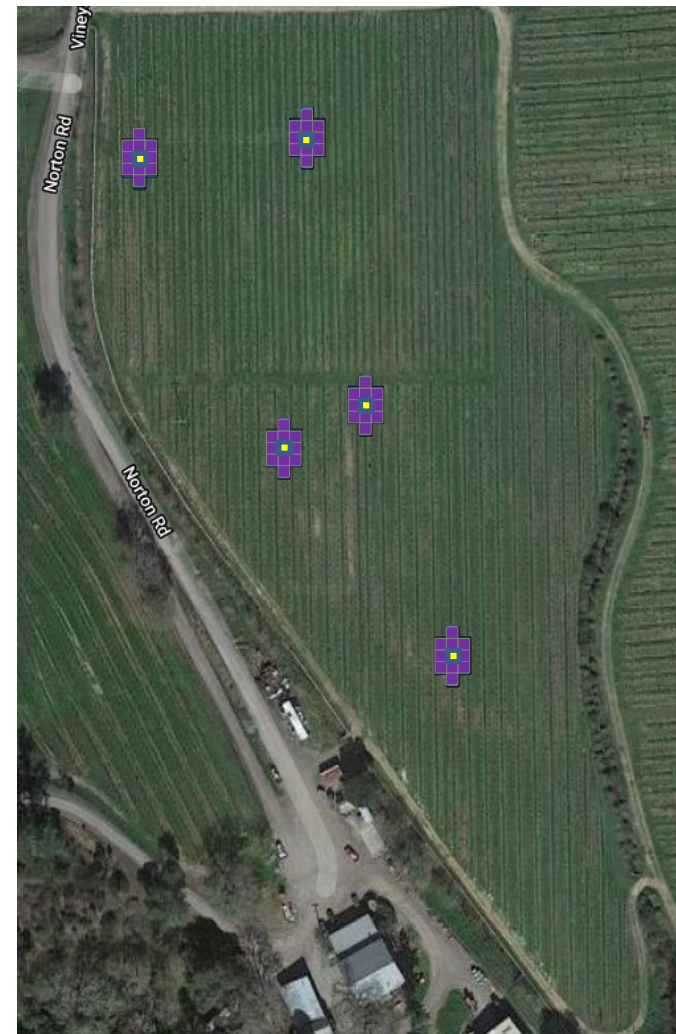
Precision Spot Treatment Program

Vines with Pierce's Disease symptoms



In areas with a few symptomatic vines over a large area

XylPhi-PD[®] suggested treatment area



Precisely treat symptomatic vines and their surrounding neighbors.

Entire Block Treatment Program

Vines with Pierce's Disease symptoms

XylPhi-PD[®] suggested treatment area

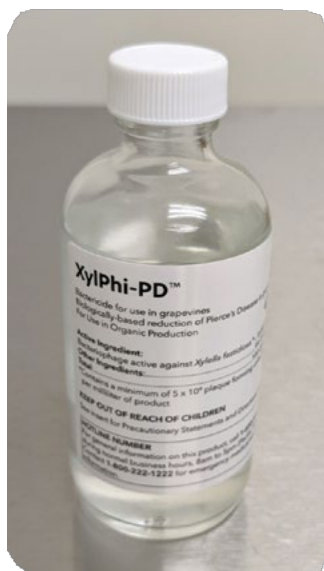


In large areas with a significant number of symptomatic vines throughout, especially if in an area where GWSS are the vector

Treat entire block to reduce PD pressure.

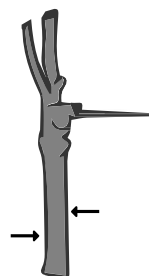
How much to apply

1



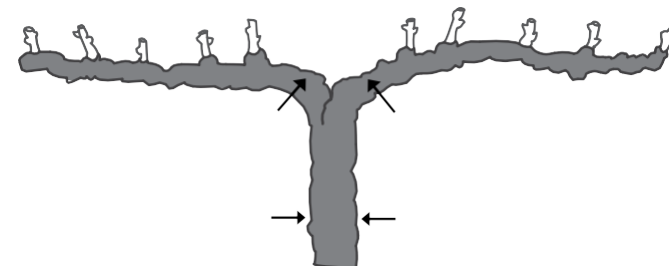
~600
replants/young vines

=



or

~300
mature vines



- A 100-mL vial of XylPhi-PD[®] treats about 600 replanted vines or 300 mature vines per application.
 - One *replant* vine application = two 0.08-mL injections/vine (0.16 mL total/application)
 - One *mature* vine application = four 0.08-mL injections/vine (0.32 mL total/application)
- Apply at least twice per season.

<i>Volume used per application:</i>	Trunk injections	Cordon injections	mL/ injection	mL/ app/vine	Vines/ vial/app
Replants / new vines	2		0.08	0.16	625
Mature vines	2	2	0.08	0.32	313

Industry reception

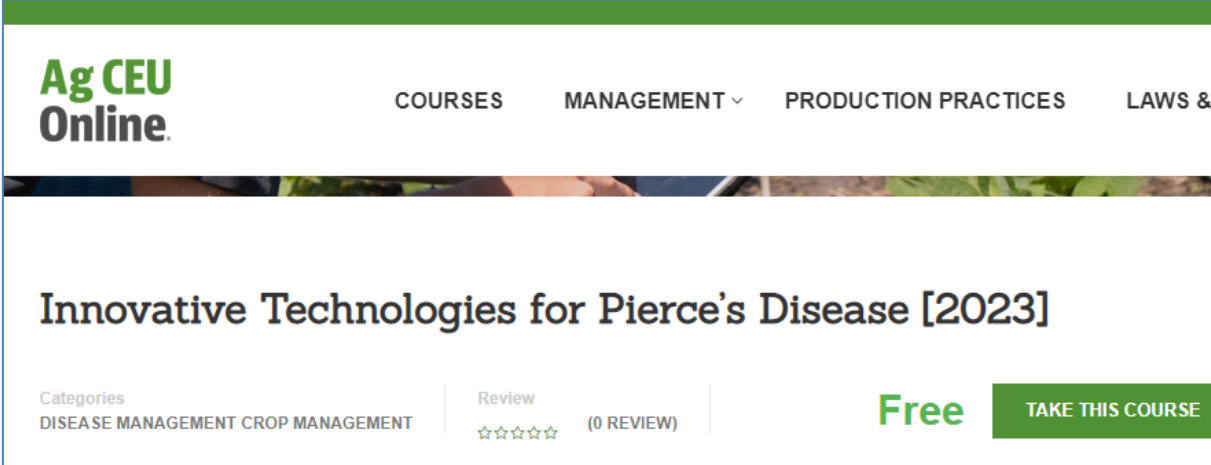


PD-infected vine

- Over 30 commercial sites currently using XylPhi-PD®
- Over 10 sites starting 4th year of use
- Growers have reported:
 - reductions in replants;
 - recovery of yields in mildly symptomatic vines;
 - reductions in visual symptoms of PD;
 - increased PD incidence in untreated areas.



Vine protected by XylPhi-PD®



Ag CEU
Online

COURSES MANAGEMENT ▾ PRODUCTION PRACTICES LAWS & P

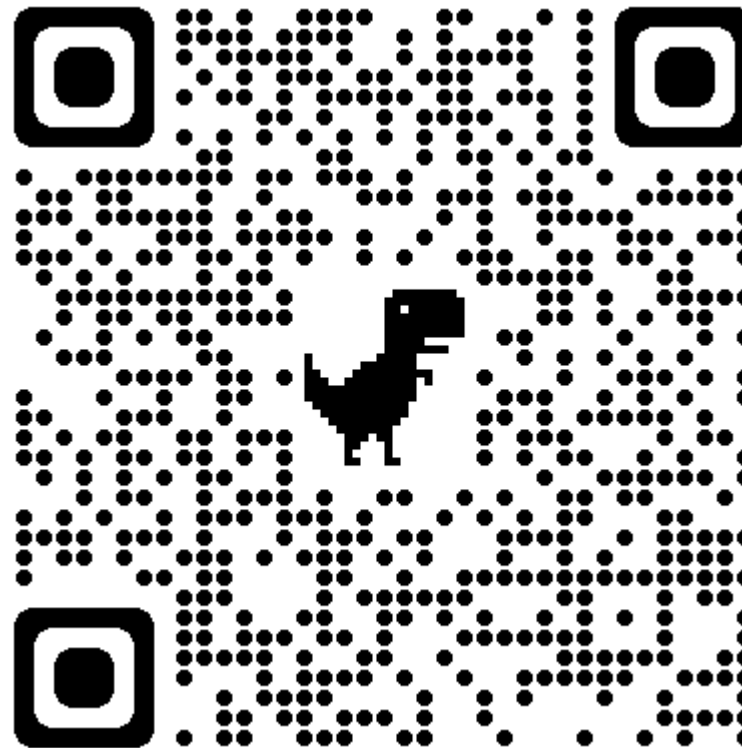
Innovative Technologies for Pierce's Disease [2023]

Categories
DISEASE MANAGEMENT CROP MANAGEMENT

Review
☆☆☆☆☆ (0 REVIEW)

Free TAKE THIS COURSE

**FREE
Continuing
Education
Hour**



**UC Davis
Field Day
2022**

Any questions?

Please contact techservices@inphatec.com for questions

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XylPhi-PD[®]

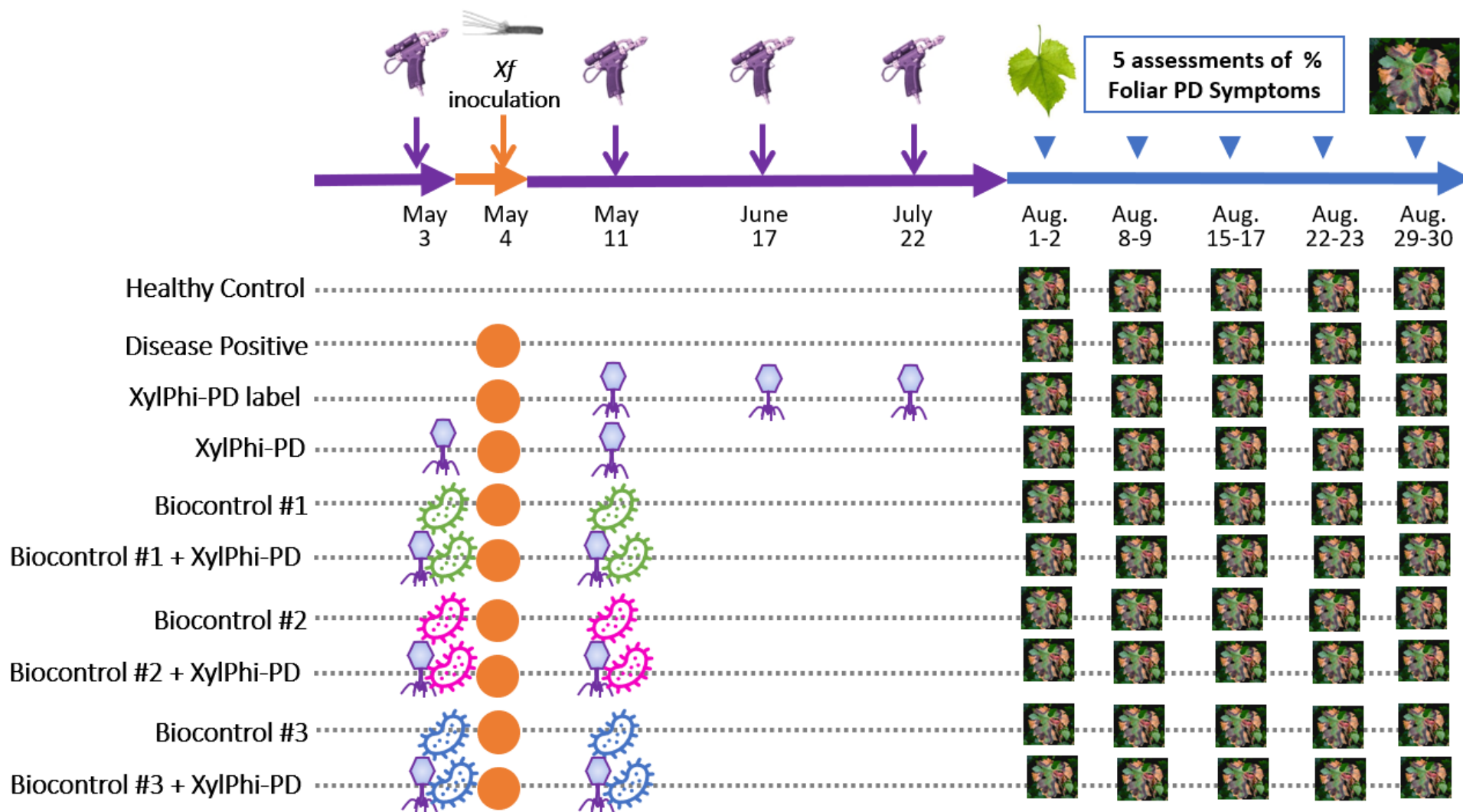
Bactericide for use in grapevines.

- The proven treatment and prevention for symptoms of Pierce's Disease.
- Alternative to costly rogueing and replacement of grapevines.
- Maintains production, efficiency, and uniformity in the block.
- Flexible application timing with durable injection system.
- No REI, minimal PPE.
- OMRI-listed for use in organic production.
- No phytotoxicity.

UC Davis Study Timeline

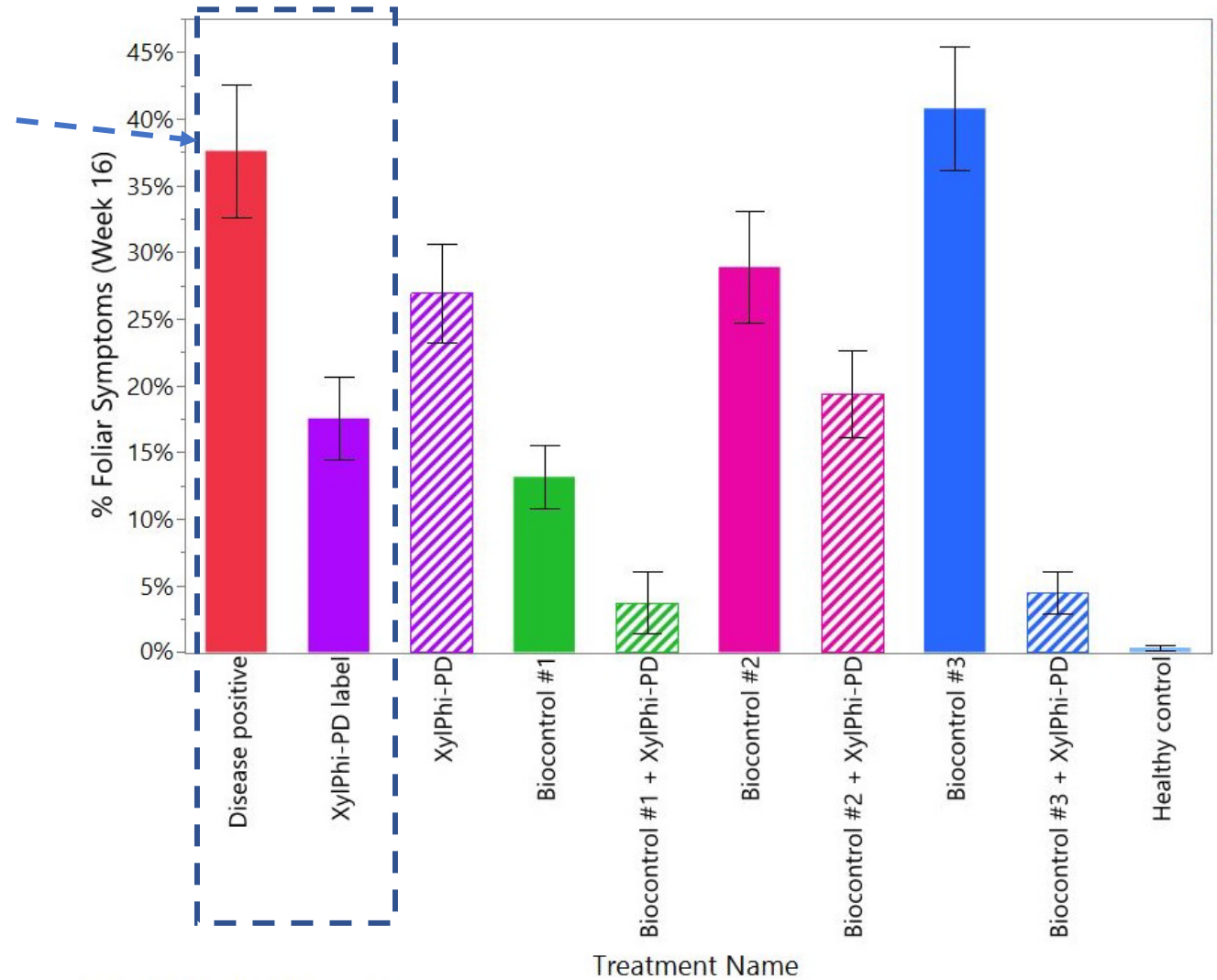
- UC Davis Armstrong Vineyard
- 11–12-year-old Cabernet Franc vines
- Stag’s Leap strain of *Xylella fastidiosa*
- Complete Randomized Block Design
- 10 vines/treatment, 4 shoots/vine

Artificial Inoculation



Efficacy of XylPhi-PD™ - UC Davis

- When XylPhi-PD™ is used per label recommendations, efficacy demonstrated as reduction in foliar symptoms
- Most treatments reduced disease symptoms at Week 16
- Increased efficacy noted for combinations of biocontrol + XylPhi-PD™ as compared to biocontrol or XylPhi-PD™ alone.



- FREE Field Day 1-hour course on agceuonline.com

Effectiveness of XylPhi-PD[®]

Commercial Grower Results

B

2022 — Russian River Valley, Guerneville, CA¹

- ~4500 Chardonnay vines replanted in 2019 (3 yr old).
- Vines along riparian area treated for 3 years in a row.
- Treated on May 26 & July 1 (80 µL, 4 inject./vine/trt)

100%

of vines still disease-free by 2022 harvest

A

2022 — Dry Creek Valley, Healdsburg, CA¹

- 400 Chardonnay 11-year-old vines (rootstock O3916) **without** symptoms of PD.
- Typical incoming infection pressure about 5% new infections/year (20/400).
- Treated on May 26 & July 25 (60 µL, 4 inject./vine/trt)

100%

of vines still symptom-free by harvest

C

2019-21 — North Coast, CA¹

- 4 vineyards with high-PD hotspots.
- 200 vines treated for 3 years, and 200 treated for 2 years.

55%

reduction of detectable Xf for all vines (including chronic/advanced cases)

84%

prevention efficacy, with no new PD cases in the 3-year treated group, only 1 case in 2-year treated group

Effectiveness of XylPhi-PD[®]

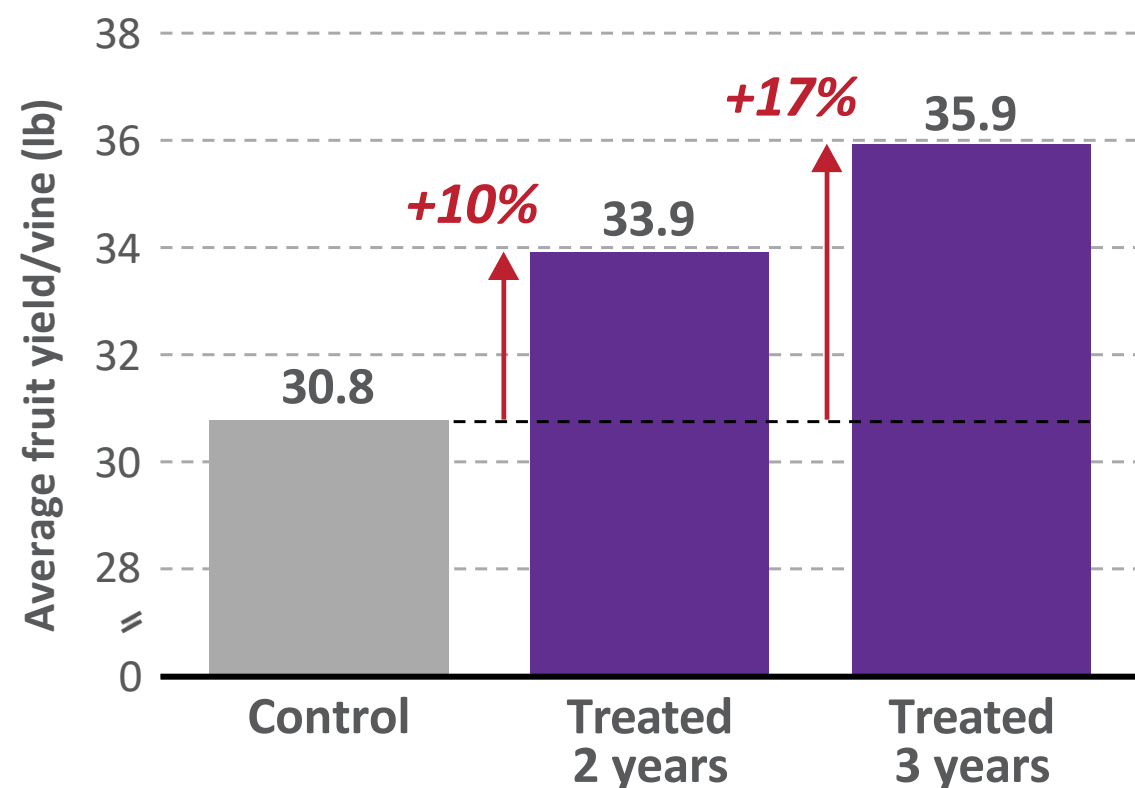
Commercial Grower Results

2021 Fruit Yield — Sonoma County, CA¹

- 8-10-year-old Chardonnay average fruit yield/vine measured.
- Vines treated for 2 or 3 years in a row.

10-17%

average increase in *fruit yield/vine* for groups treated 2 years and 3 years



1. Data on file. A&P Inphatec.

Effectiveness of XylPhi-PD[®]

□ *University Research*

A 2015 — Texas A&M greenhouse study²

- Vines inoculated with *Xf*.
- Treated vines compared to non-treated controls.

87%

reduction in symptoms of PD

B 2017 — California university field trial³

- Vines in a vineyard inoculated with *Xf*.
- Treated vines compared to non-treated controls.

80%

reduction in symptoms of PD at 7 weeks after 3rd application

C 2022 — UC Davis biocontrol study⁴

- Vines inoculated with *Xf*.
- **Severe challenge infection**, with 37% of foliage showing symptoms.

53%

reduction in symptoms of PD under extraordinarily severe infection conditions

². Texas A&M Research Progress Report, 2015. Data on file.

³. Regulatory filings to CDPR: Otsuka - OPC-821 Submission to DPR - Oct 31 2018.pdf.

⁴. Project 00-032-V, Innovative technologies for Pierce's Disease field day, <https://agceonline.com/299/course/Innovative-Technologies-for-Pierces-Disease-2023>.