Detailed studies of plant pathogens can lead to novel means of disese control: The case of *Xylella fastidiosa* in grape and citrus

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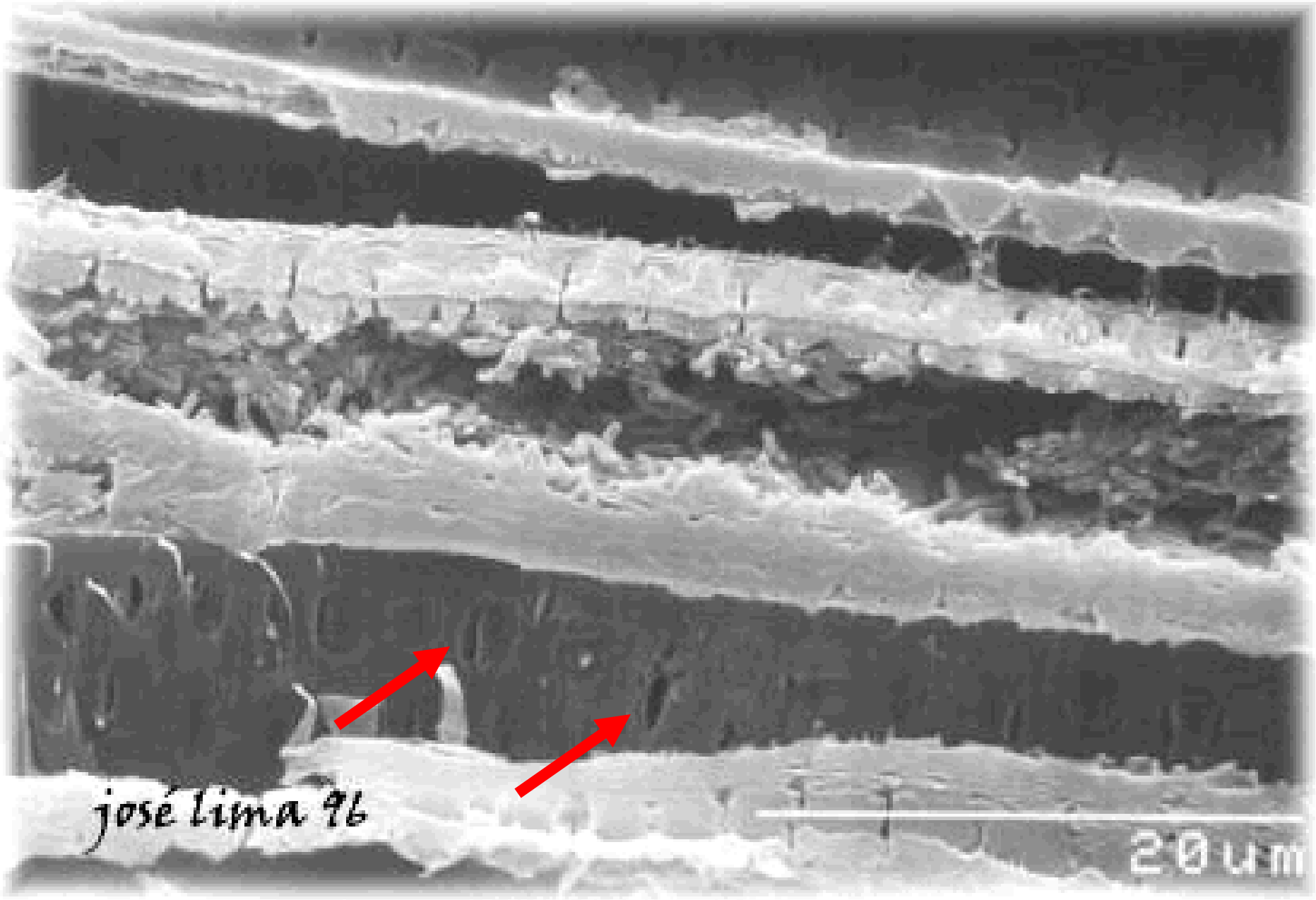
Xylella fastidiosa is obligately vectored by various xylem-feeding insects

Blue-green sharpshooter
Graphocephala atropunctata

Glassy-winged sharpshooter
Homalodisca coagulata

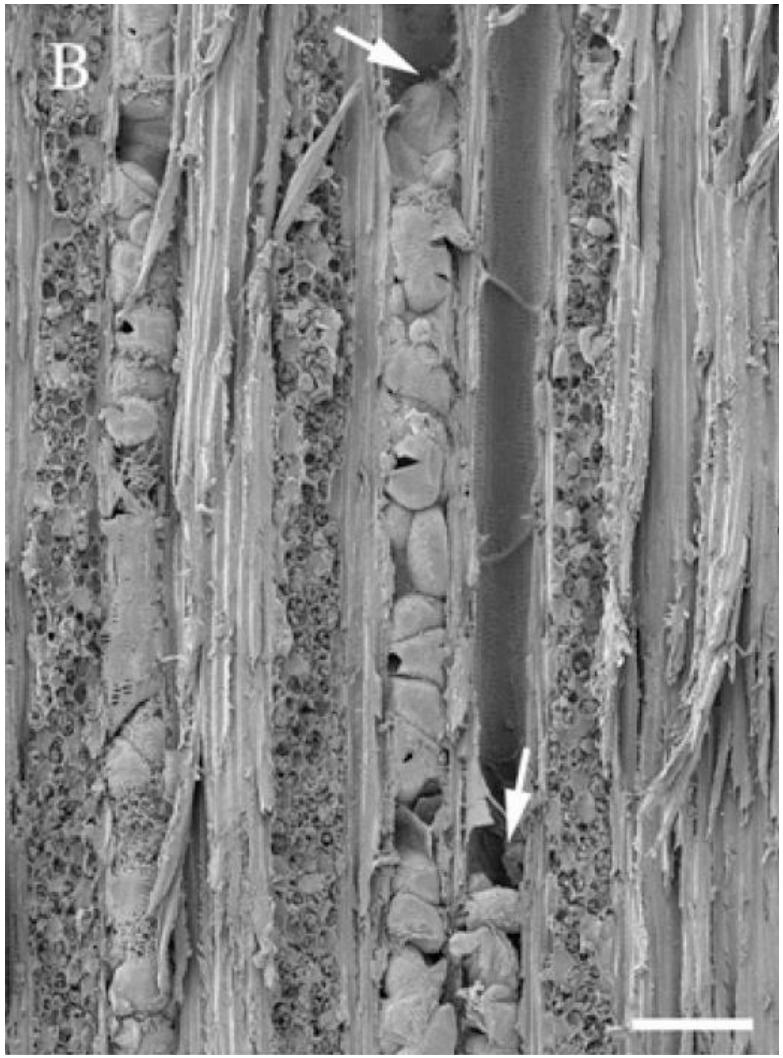
Xylem pits enable by-pass of water around blocked vessels



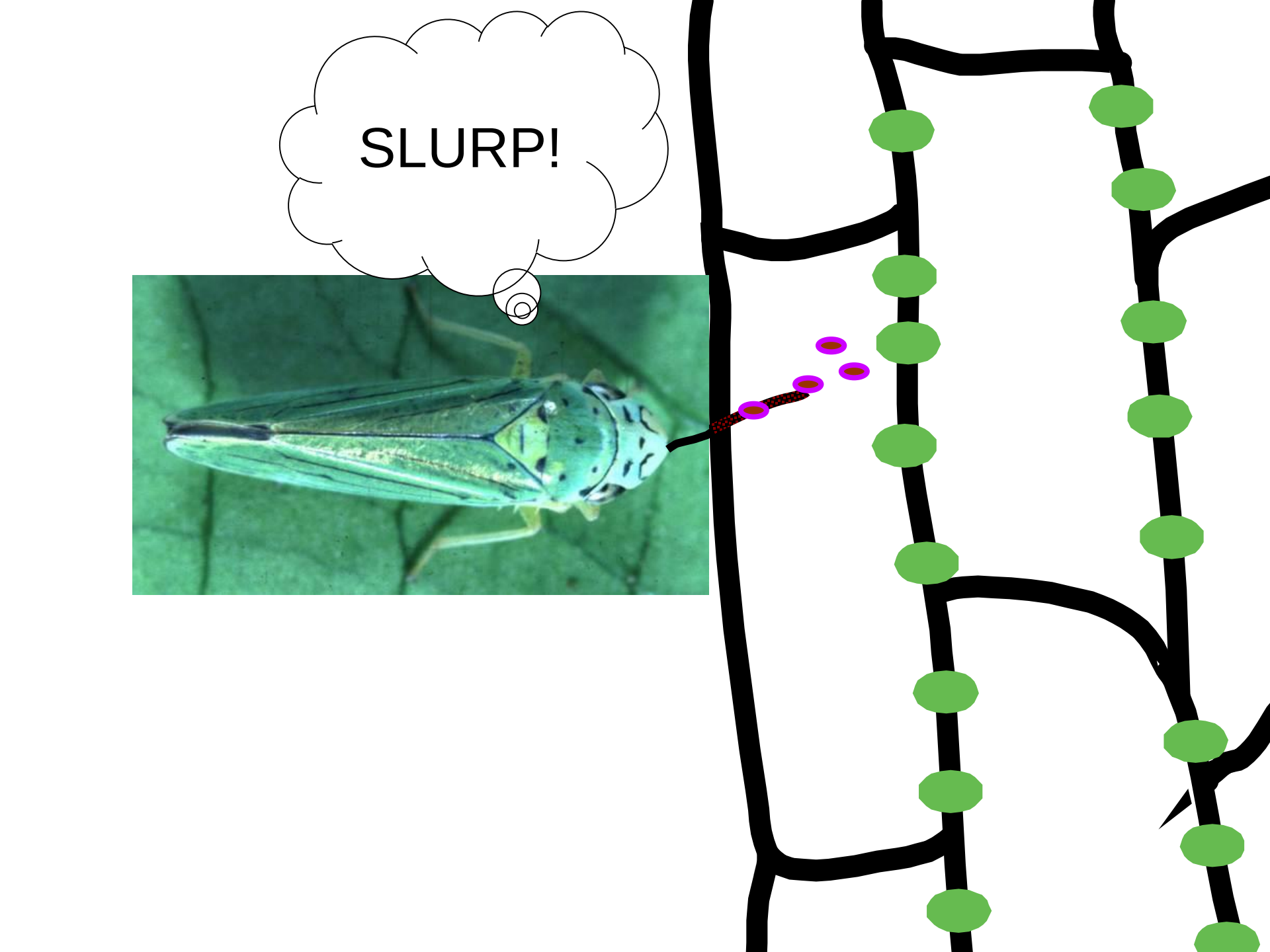
Vascular Occlusions in Grapevines with Pierce's Disease Make Disease Symptom Development Worse

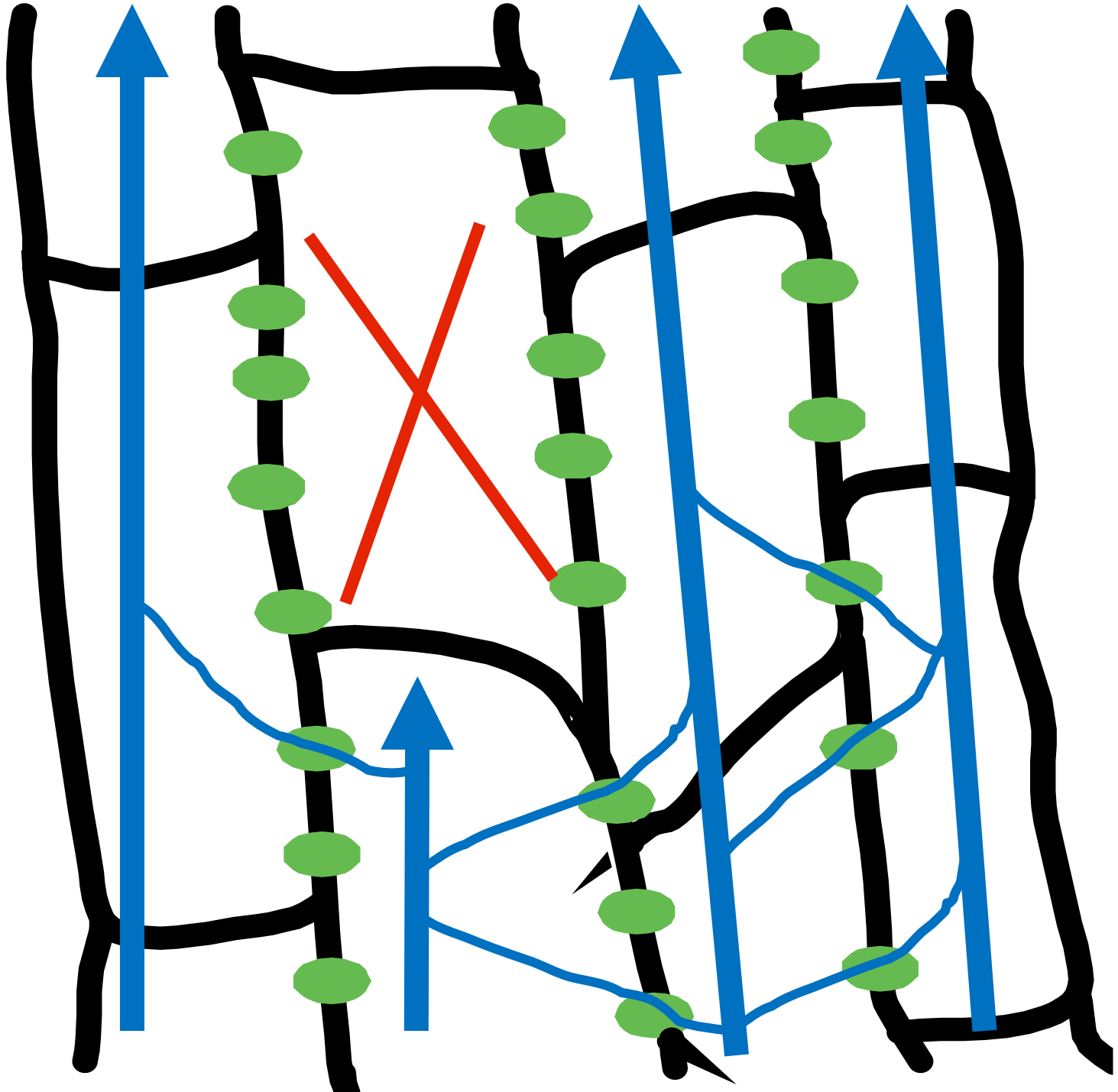
Qiang Sun*, Yuliang Sun, M. Andrew Walker, and John M. Labavitch

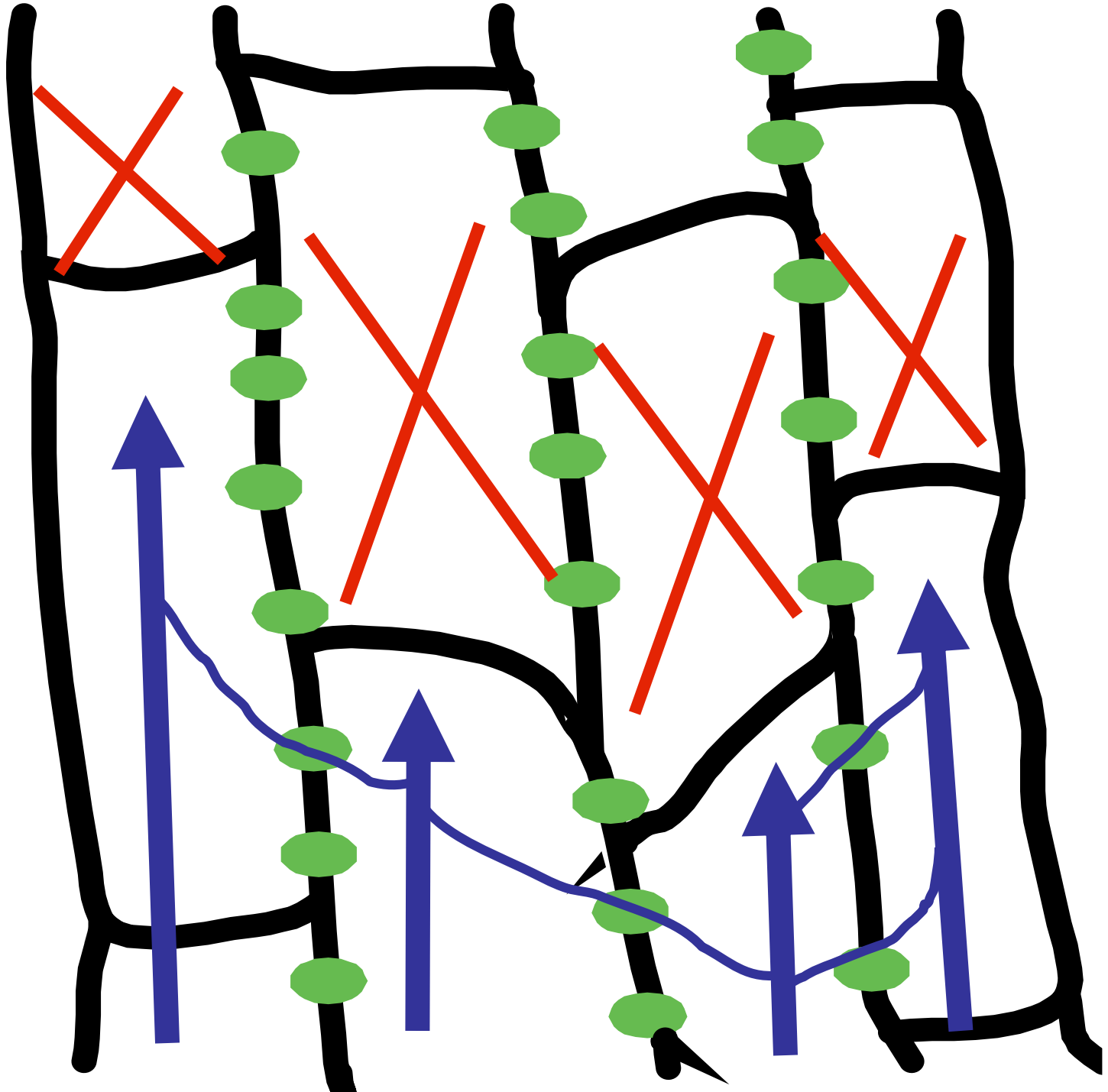
Plant Physiology 161:1529 (2013)



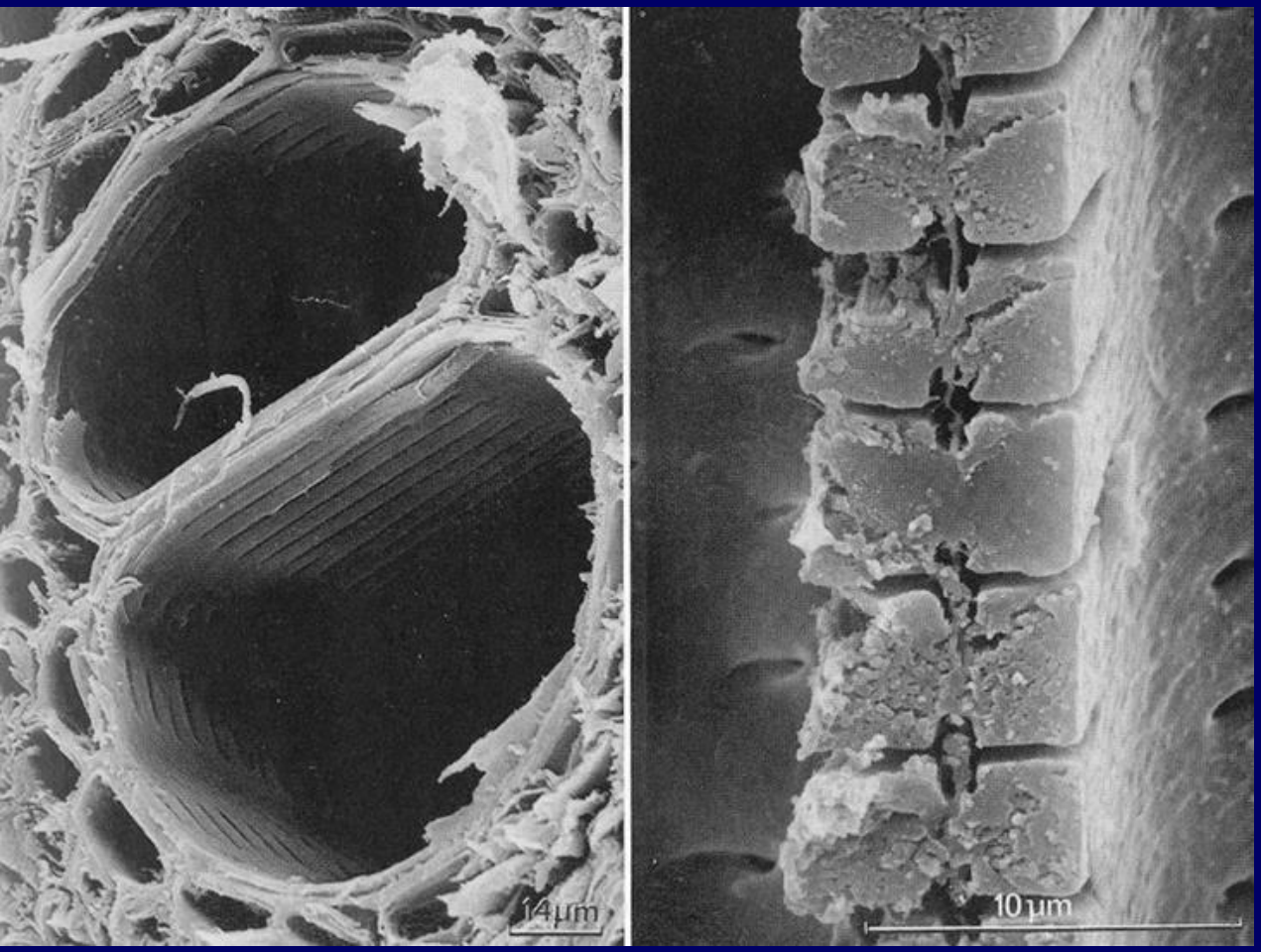
Many/most plants do not excessively react to *X. fastidiosa*?


SLURP!

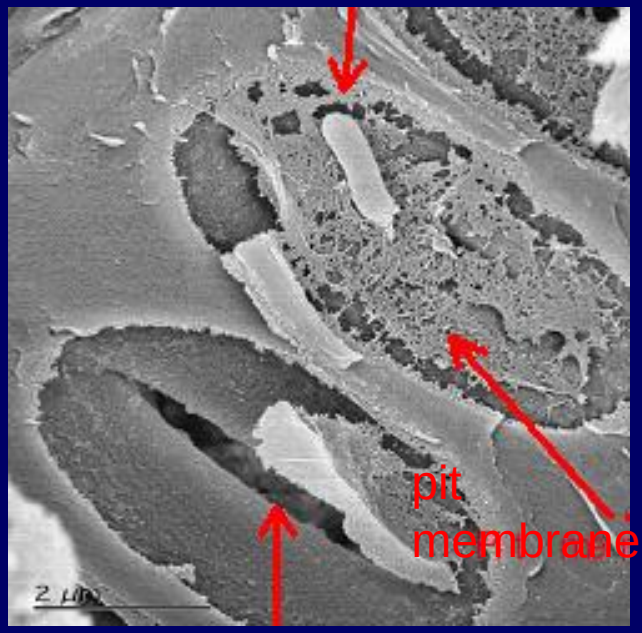




Access from vessel to vessel through bordered pits is blocked by the pit membrane

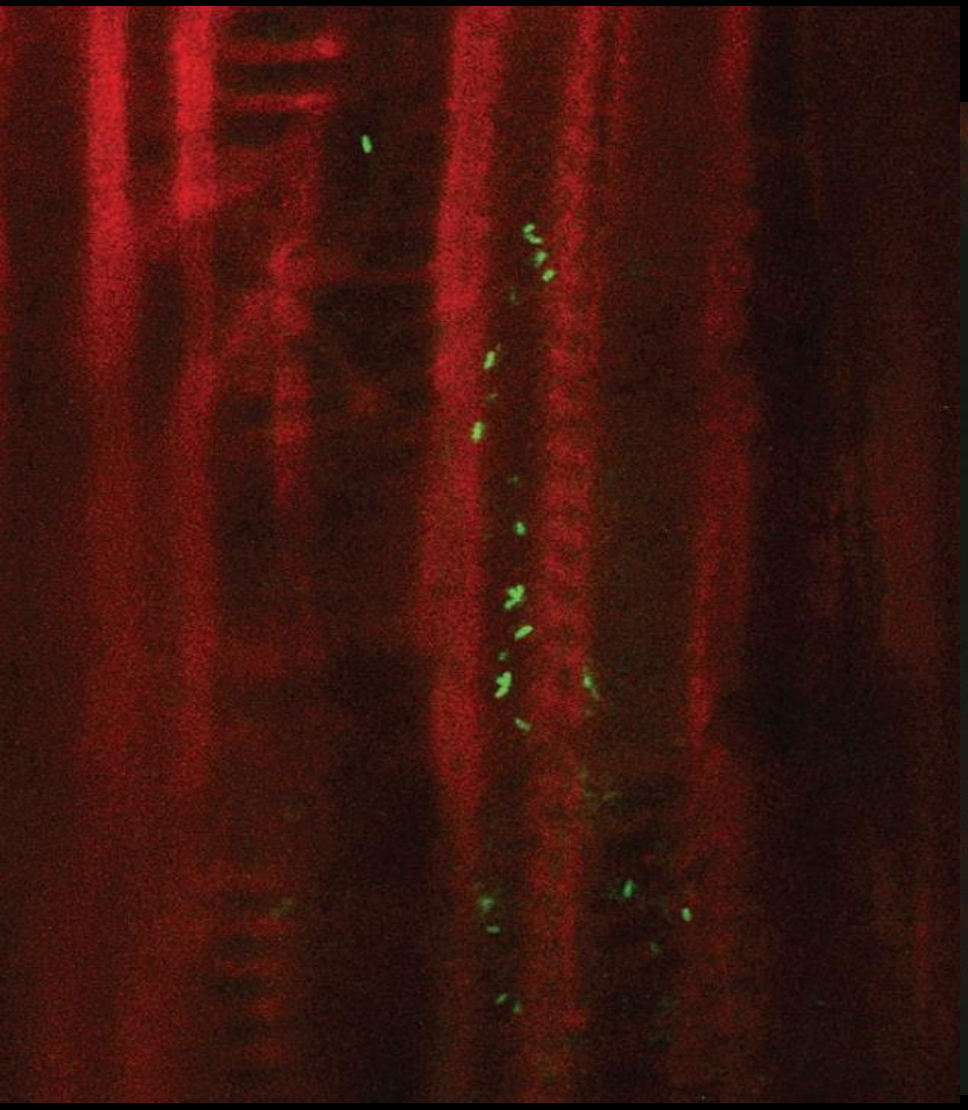


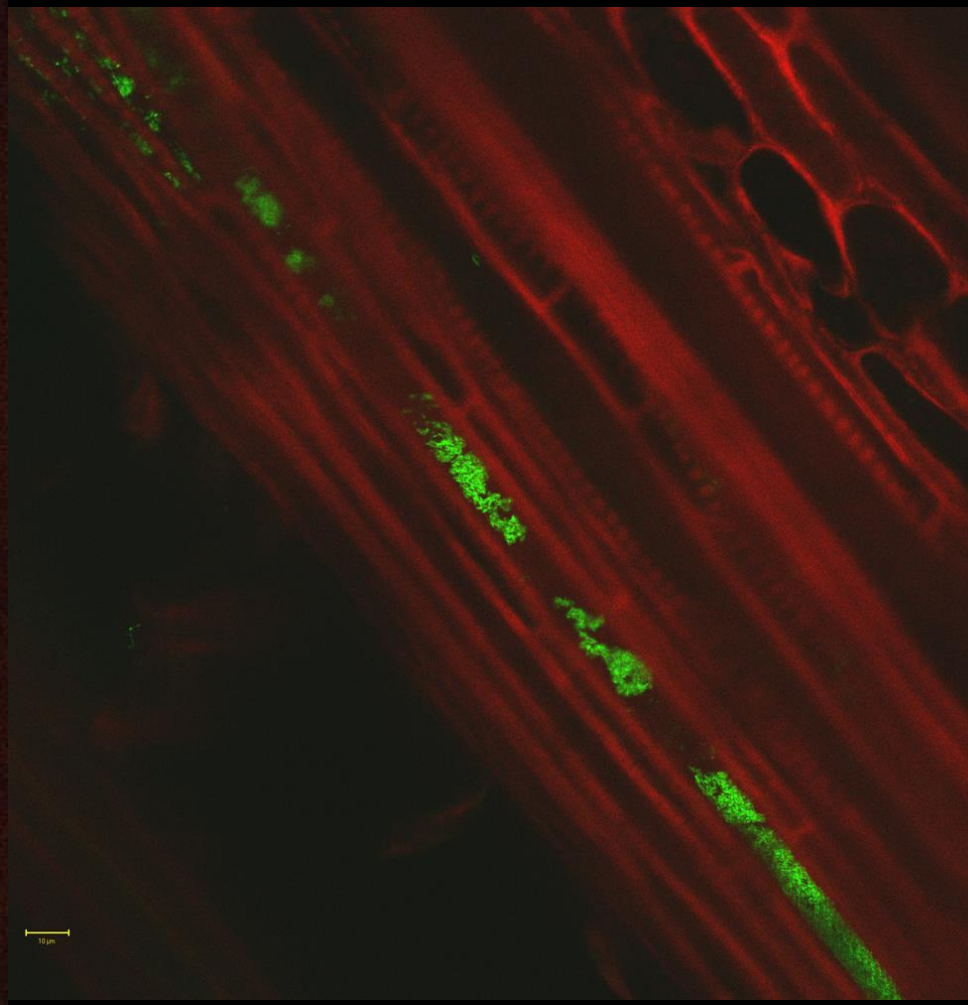
Xylella cell

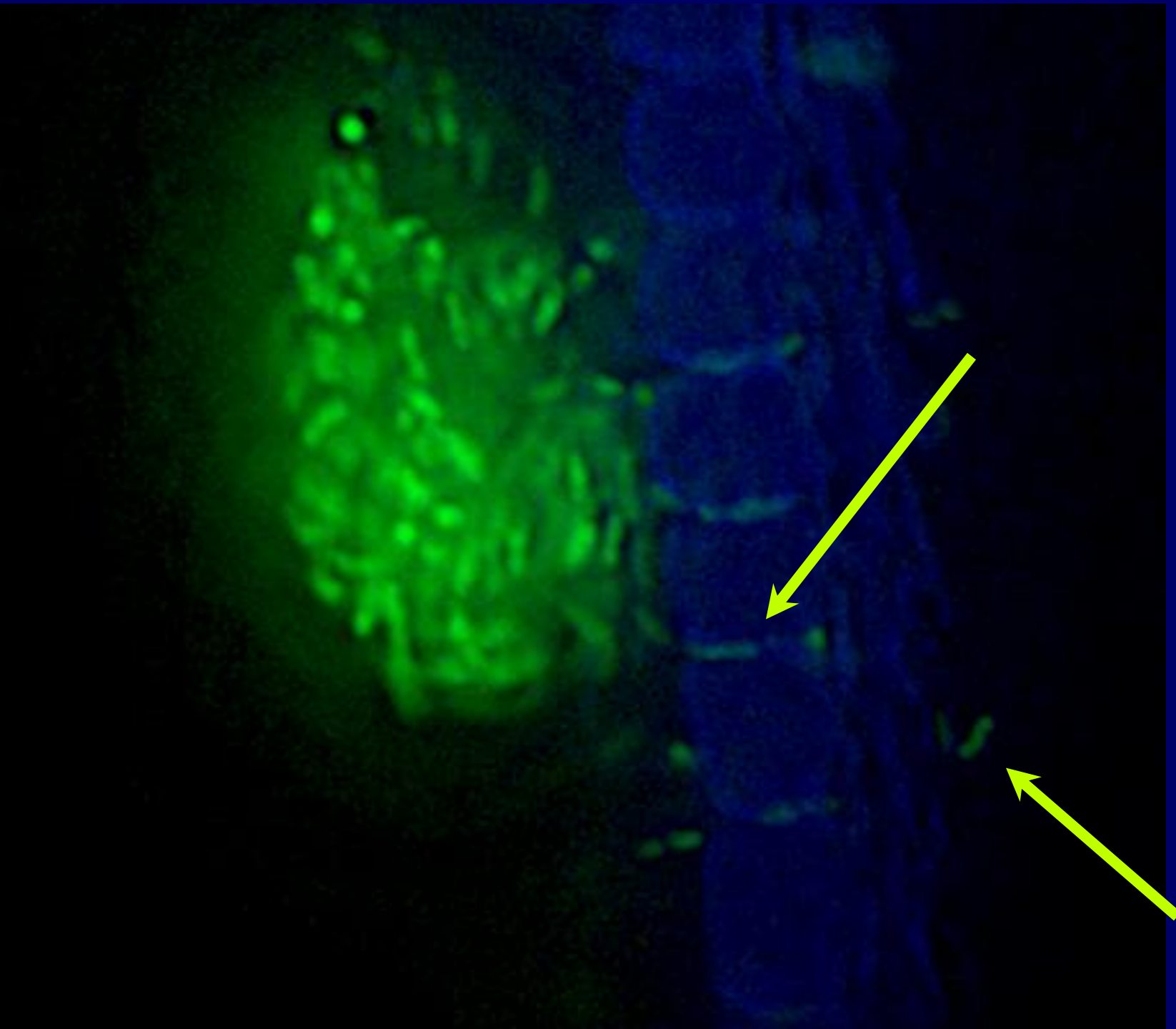


Bordered pit opening

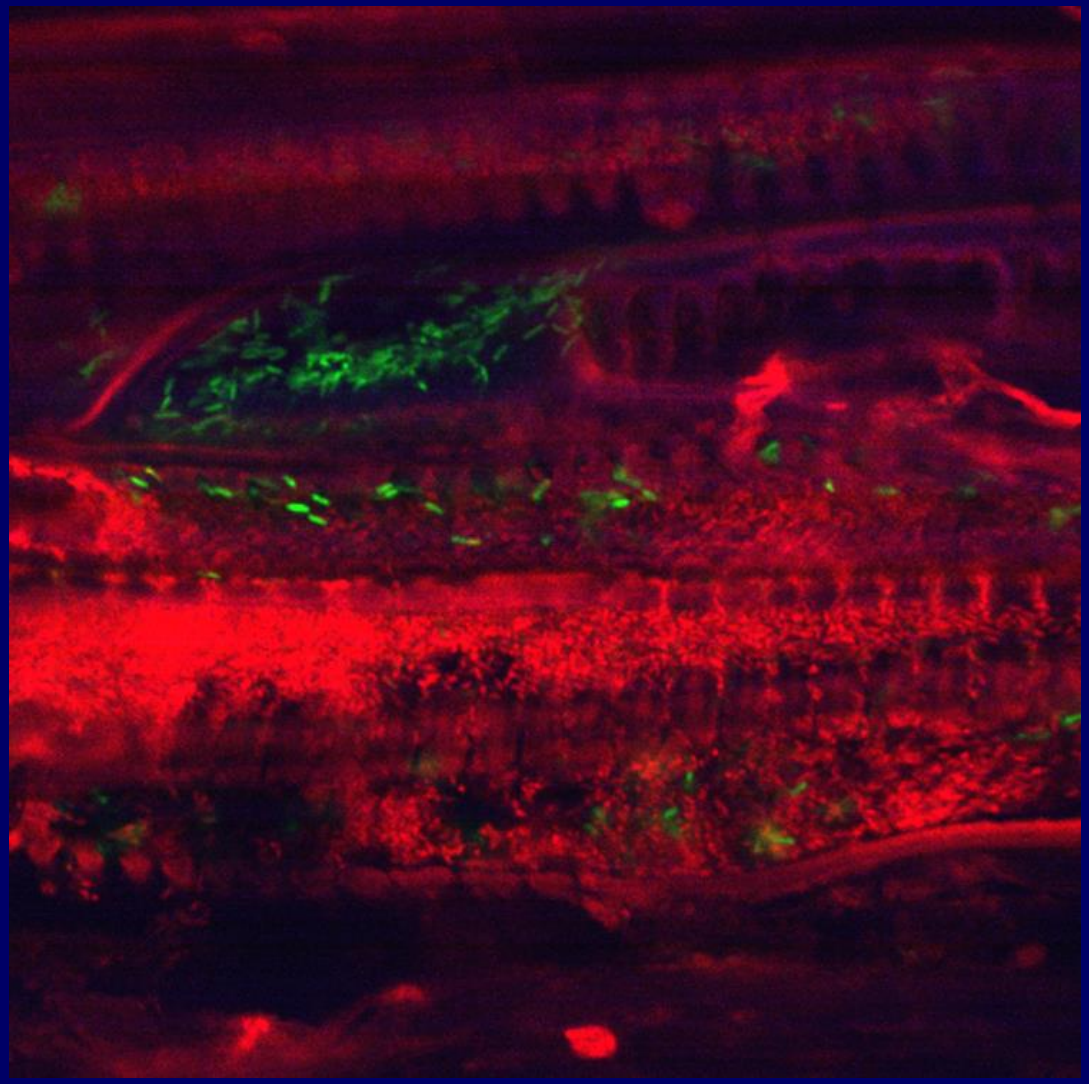
X. fastidiosa cells are most commonly found in modest sized micro-colonies within xylem vessels



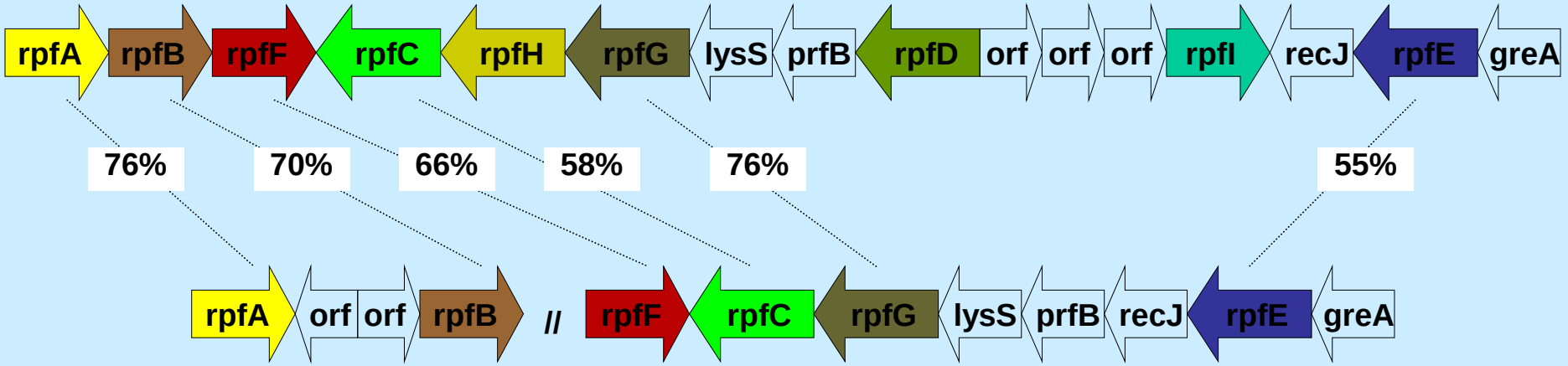




X. fastidiosa cells in heavily blocked regions of heavily infected leaves are mostly dead



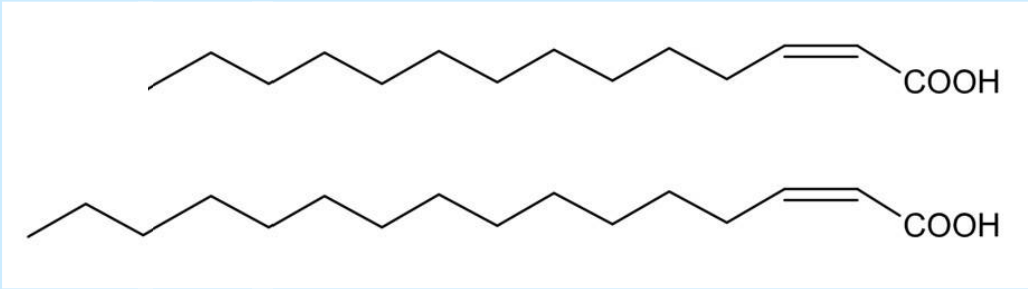
Xanthomonas campestris pv. *campestris*



Xylella fastidiosa

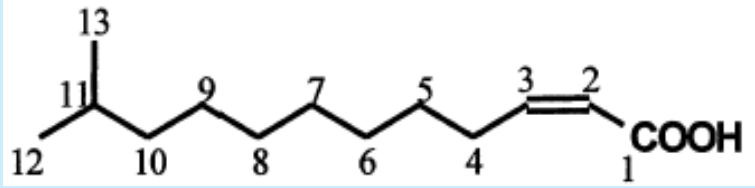
rpfB,F Synthesis of “diffusible signal factor” (DSF)

rpfG,C DSF perception and signal transduction leading to virulence trait expression



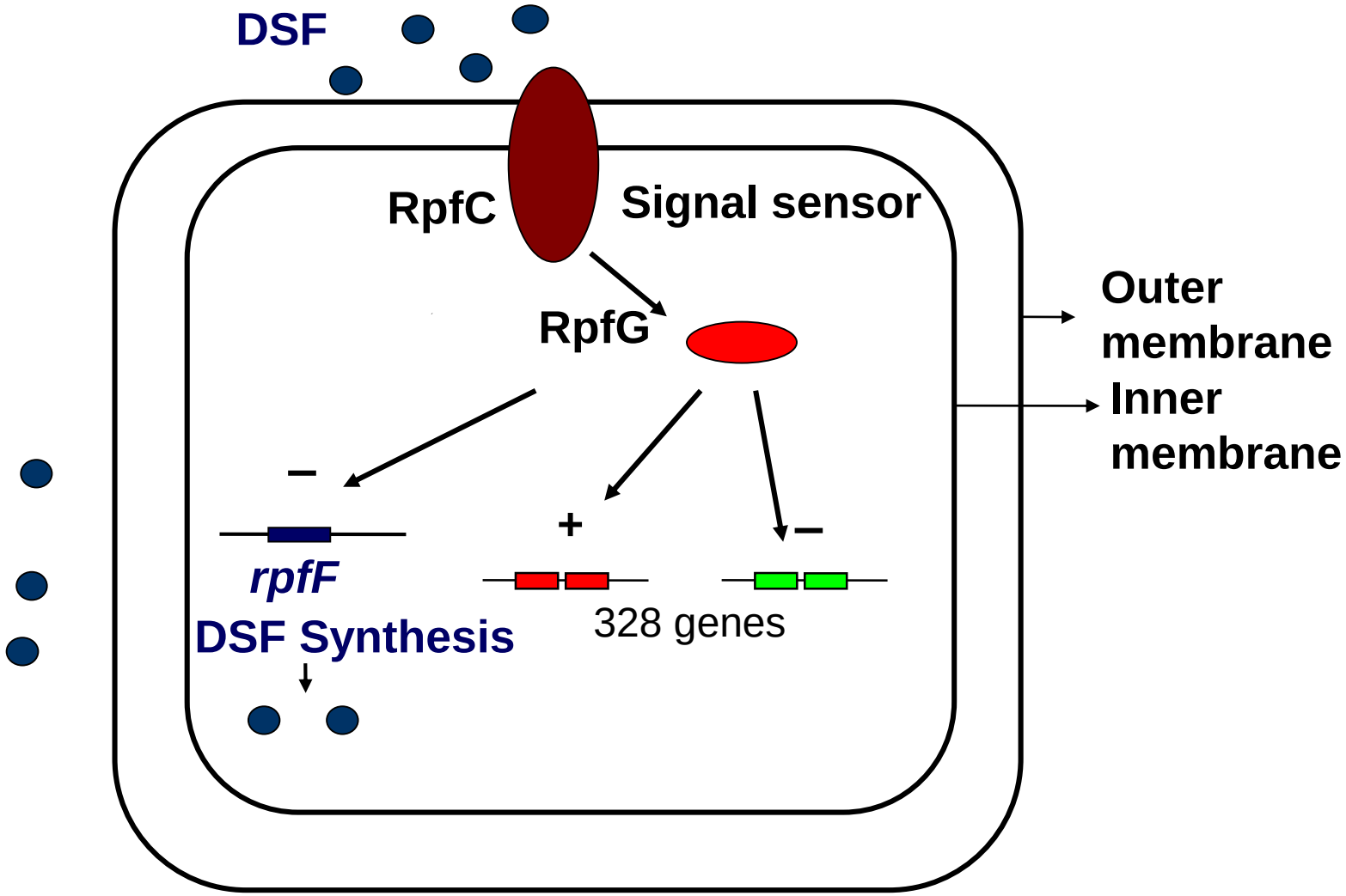
C14-cis XfDSF1

C16-cis XfDSF2



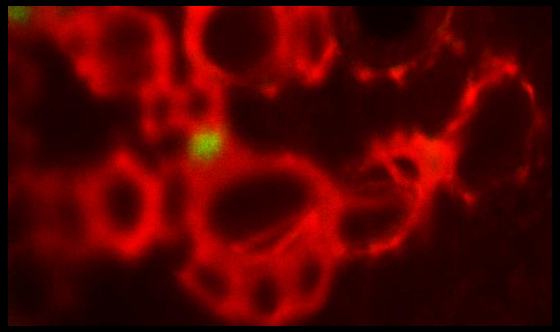
Xcc DSF

DSF-mediated cell density-dependent gene regulation in *Xylella fastidiosa*

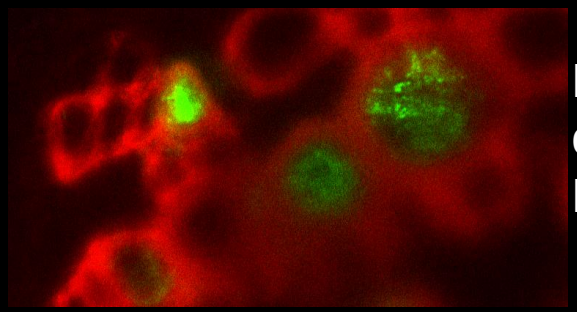


X. fastidiosa Decreases Virulence by Supressing Virulence Genes in Cell Density-Dependent Fashion

Wild type

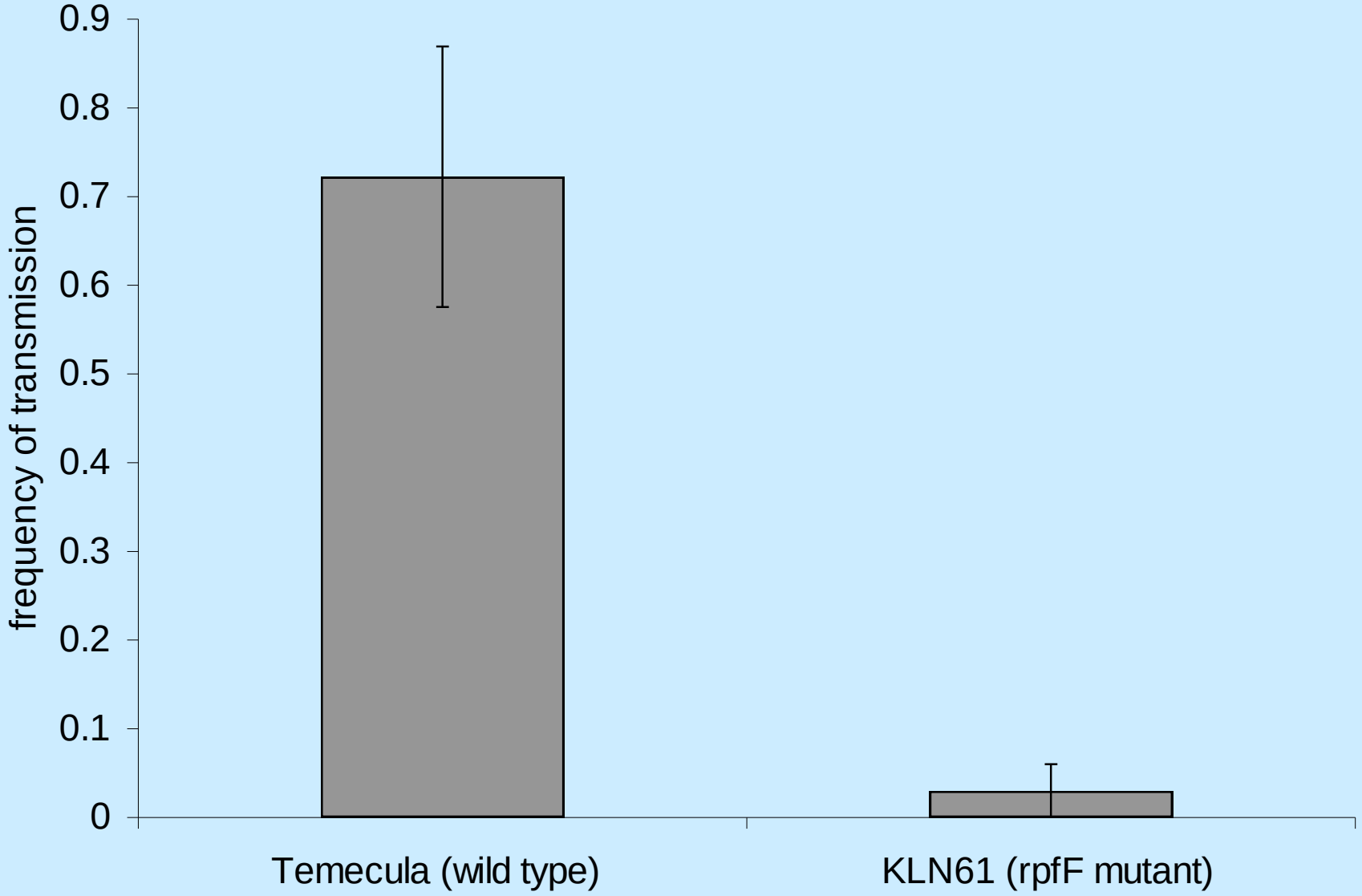


***rpfF* mutant**



More vessels colonized &
More cells/vessel

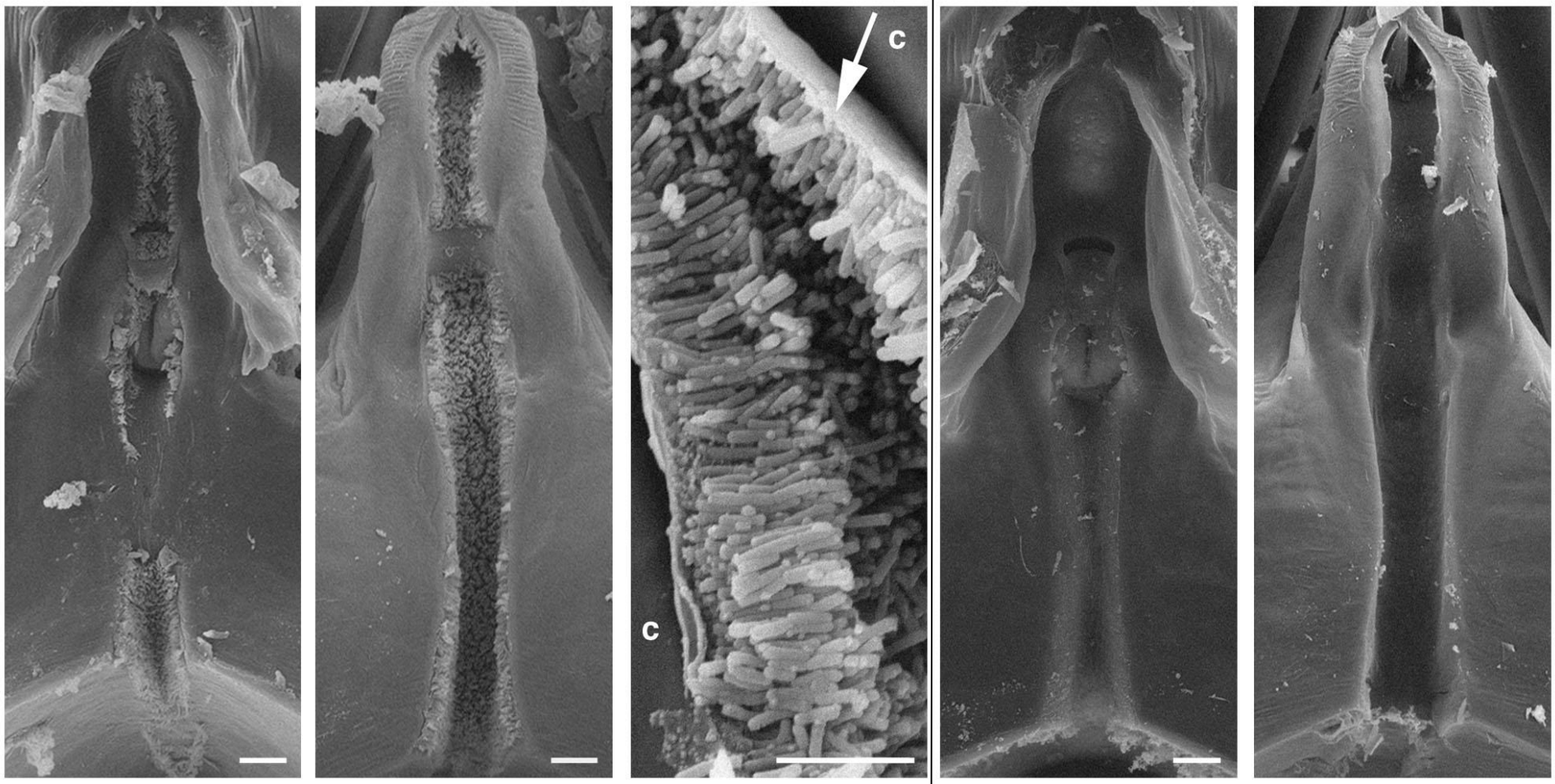
rpfF mutants, which cannot signal, are severely impaired in transmissibility by an insect vector



rpfF mutants cannot form the biofilm typical of *X. fastidiosa* colonies in insect foreguts

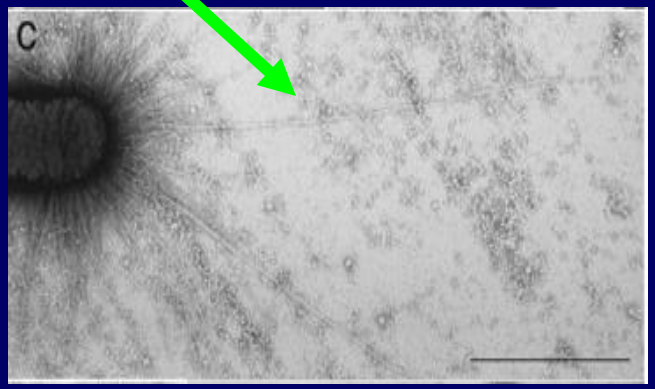
wild type

rpfF mutant



Key Virulence Genes Controlled by RpfF-mediated cell-cell signaling

Type IV pili



Meng et al. 2005

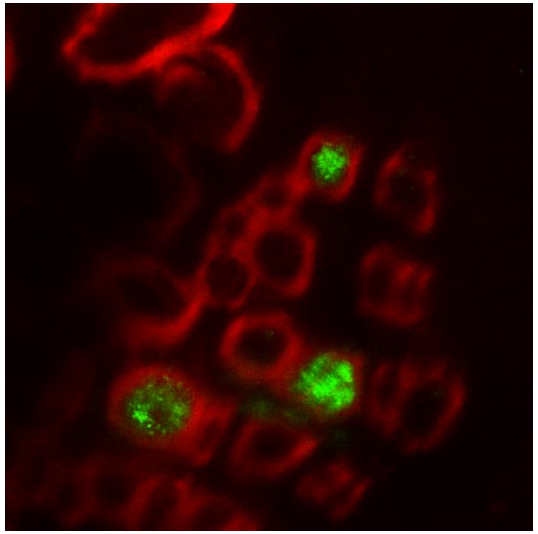
Downregulated (virulence):

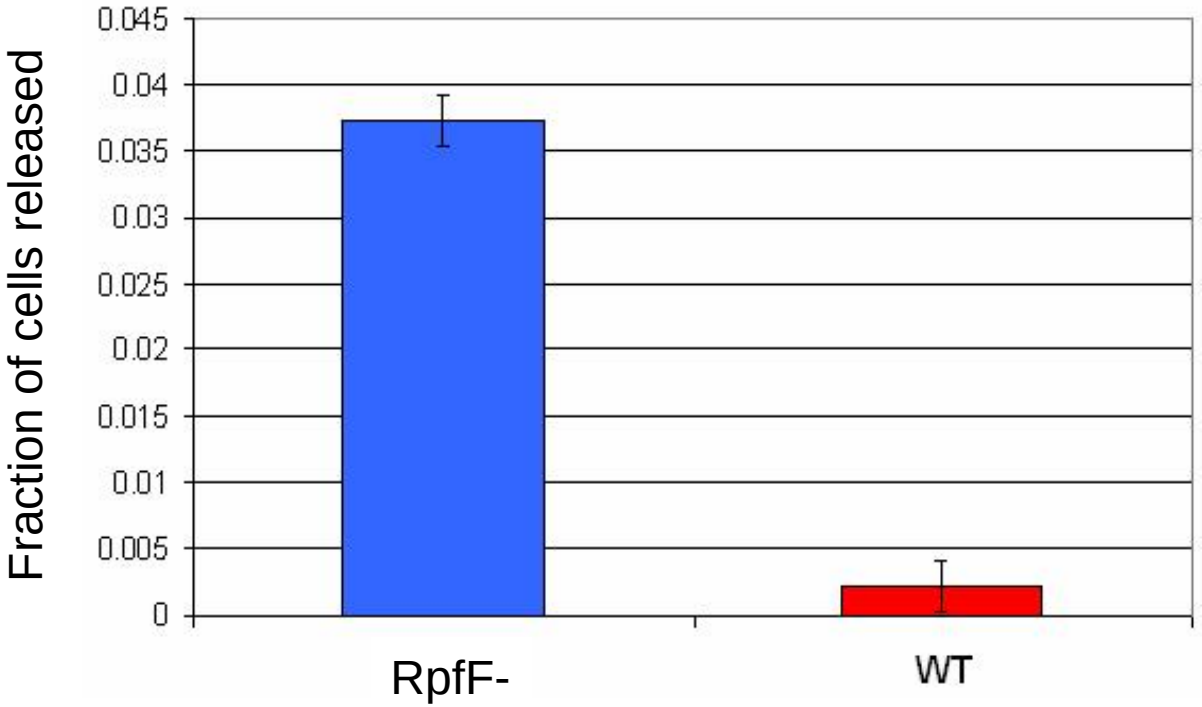
Type IV pili (twitching motility)
Polyglacturonase
Cellulase

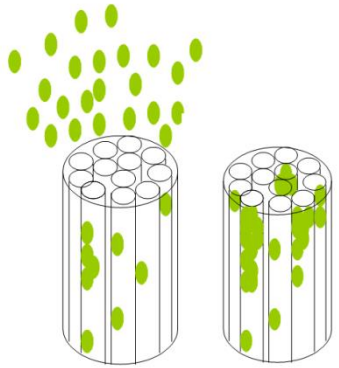
Upregulated (anti-virulence genes):

Hemagglutinin adhesins
Extracellular polysaccharides (EPS): gum
Type I pili (anchoring)

RpfF- mutants of *Xylella fastidiosa* which do not produce DSF are not “sticky” in culture – and do not adhere as tightly to plant tissue as WT strains

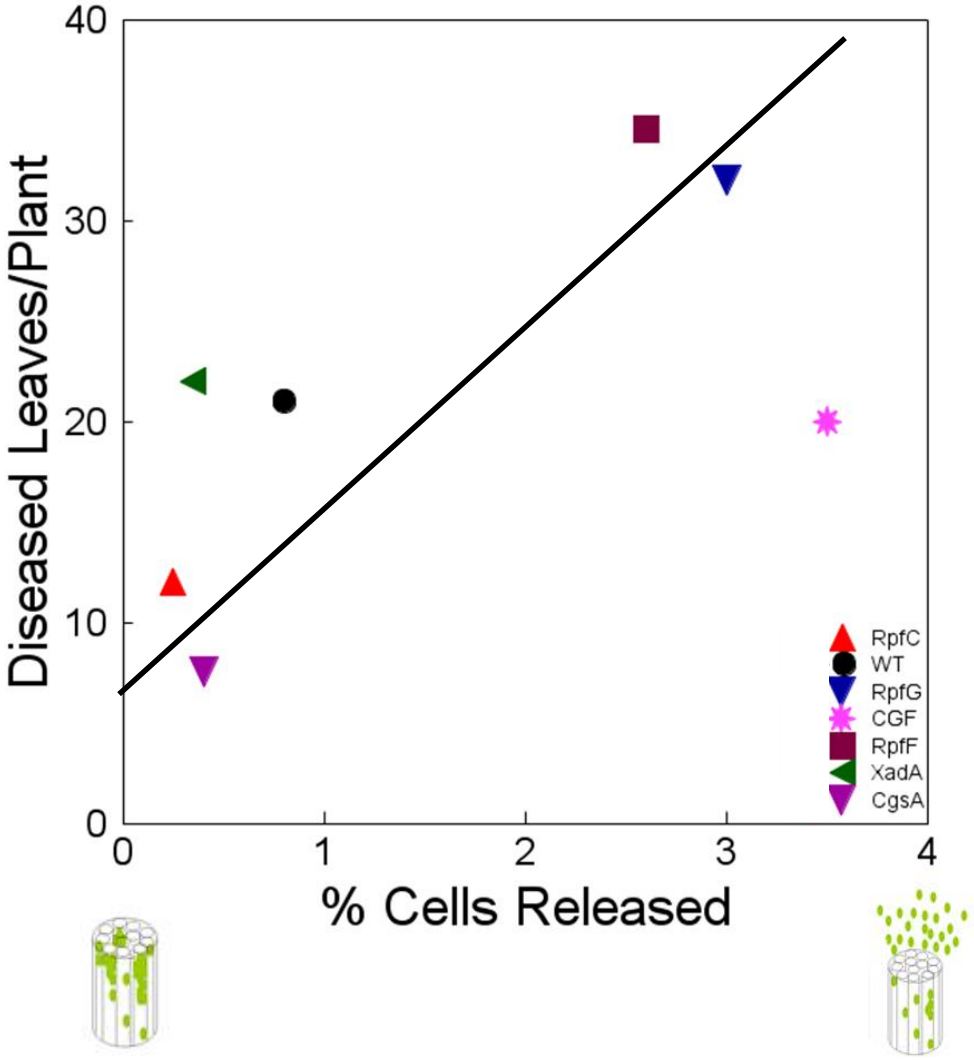




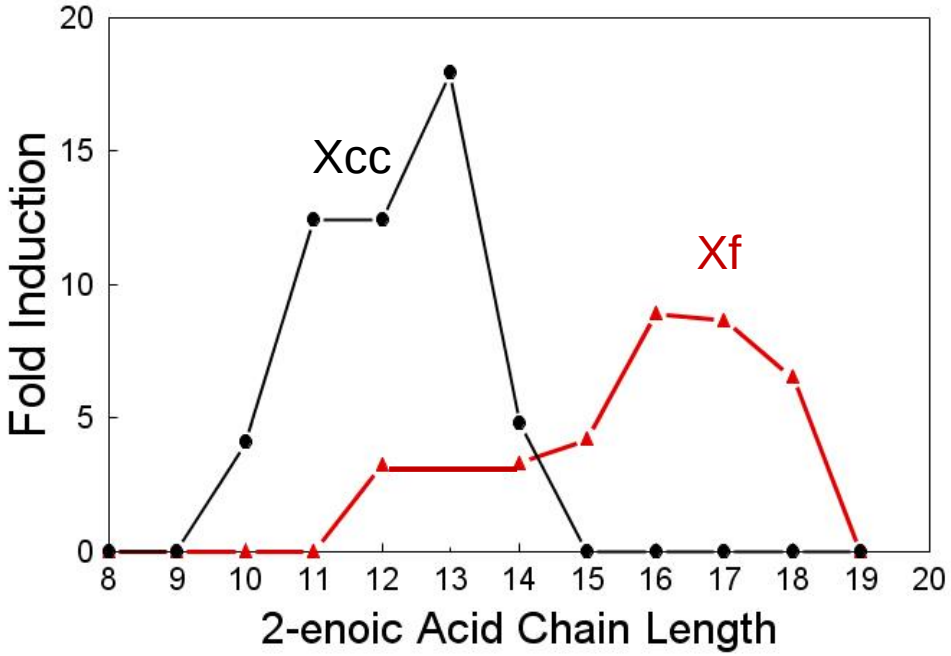


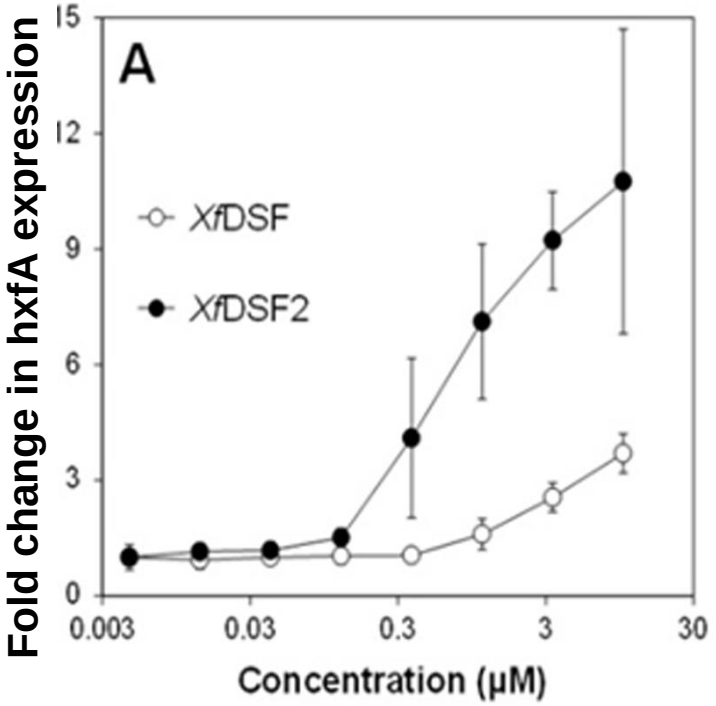
Adhesive biofilms antagonize virulence of *Xylella fastidiosa*

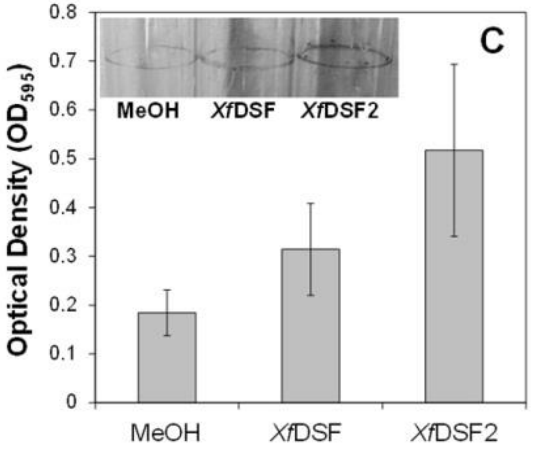
Inverse relationship between retention strength of *X. fastidiosa* to grape xylem and their virulence to grape

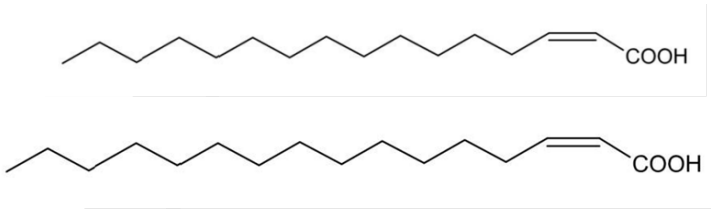


X. fastidiosa appears to have a relatively promiscuous signaling system – potentially producing and responding to various fatty acid signals – Signal production and response dependent on environment?





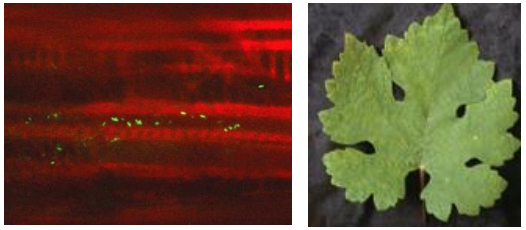




Partitioning of the population of *X. fastidiosa* for mutually incompatible processes by cell density signaling

Plant colonization phase

Active vessel colonization
Low cell numbers in most vessels
Disease symptoms may not be present



Insect acquisition phase

Some vessels have high cell numbers
Disease symptoms may be present
Further multiplication in crowded vessels slows

DSF Abundance

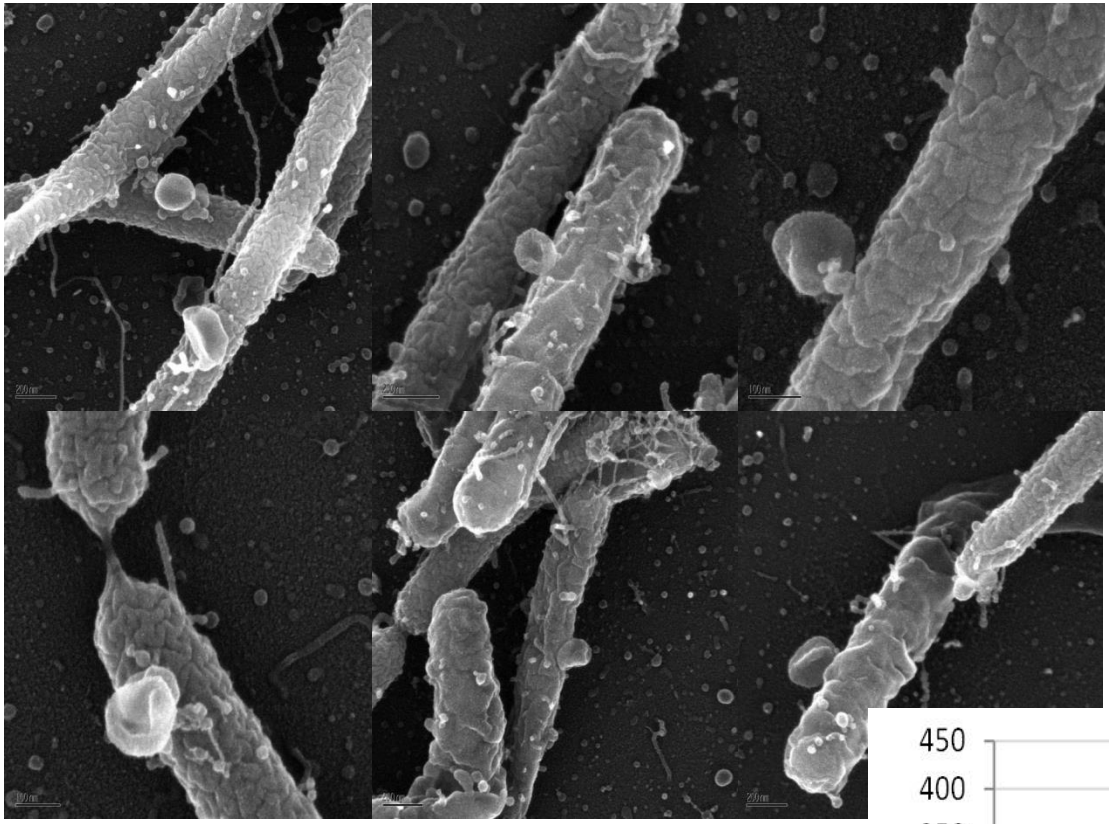
Expression of adhesins
Stickiness to Surfaces

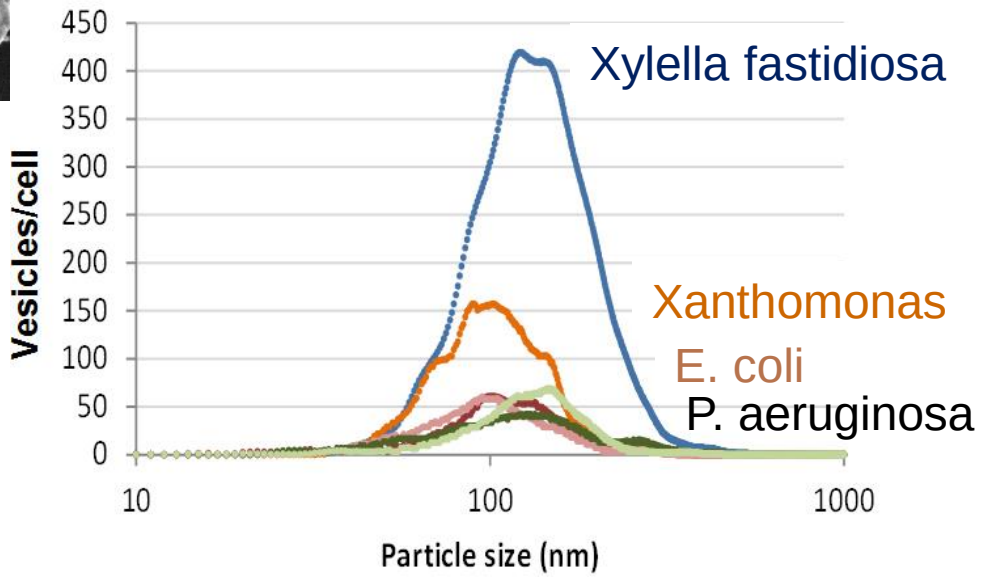
Type IV pili
Twitching Motility

Pgl and Eng expression
Pit Membrane Degradation

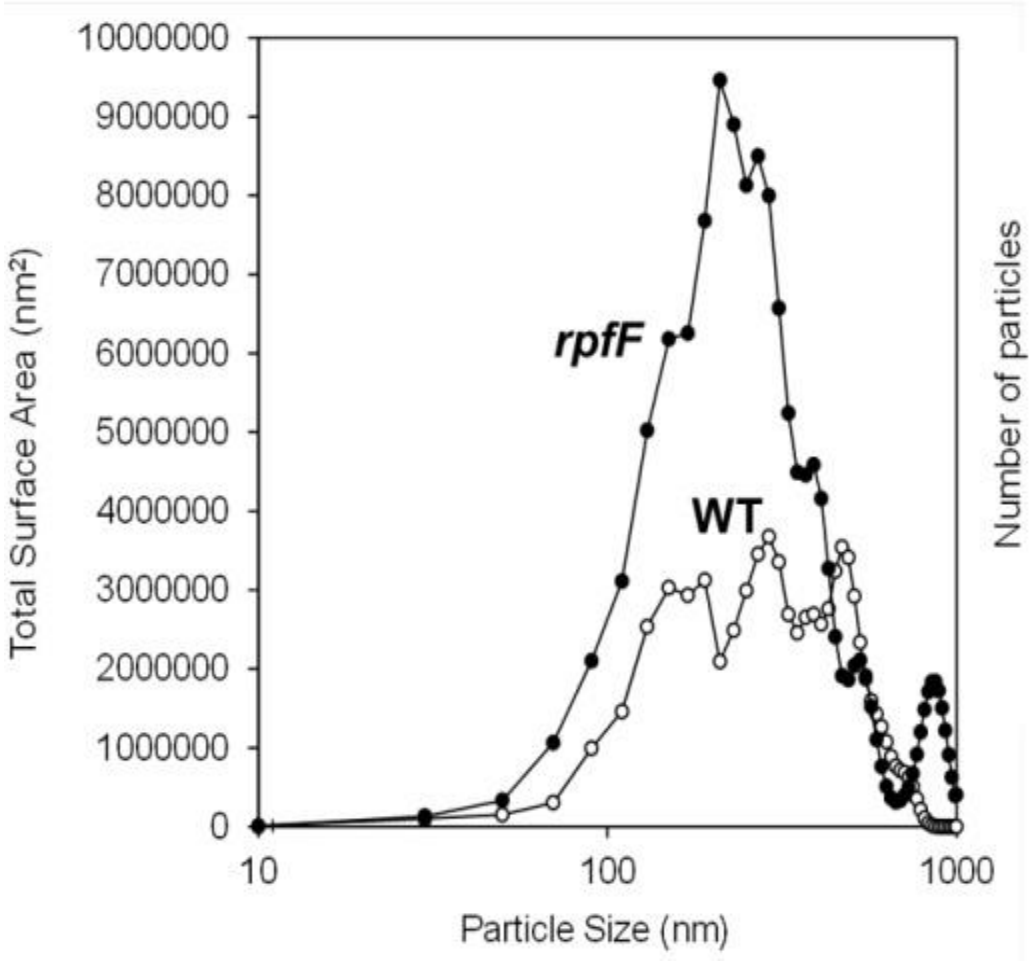
Gum Production

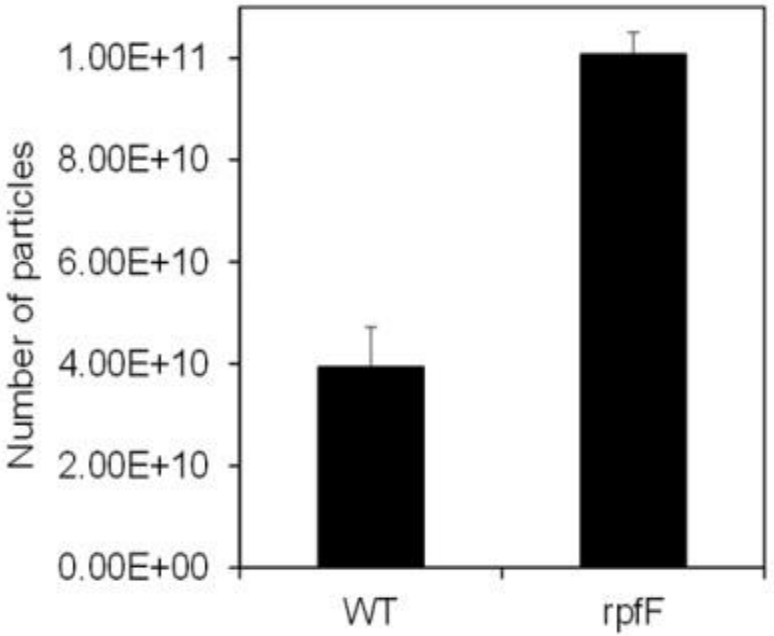
Xylella fastidiosa is a very promiscuous producer of vesicles



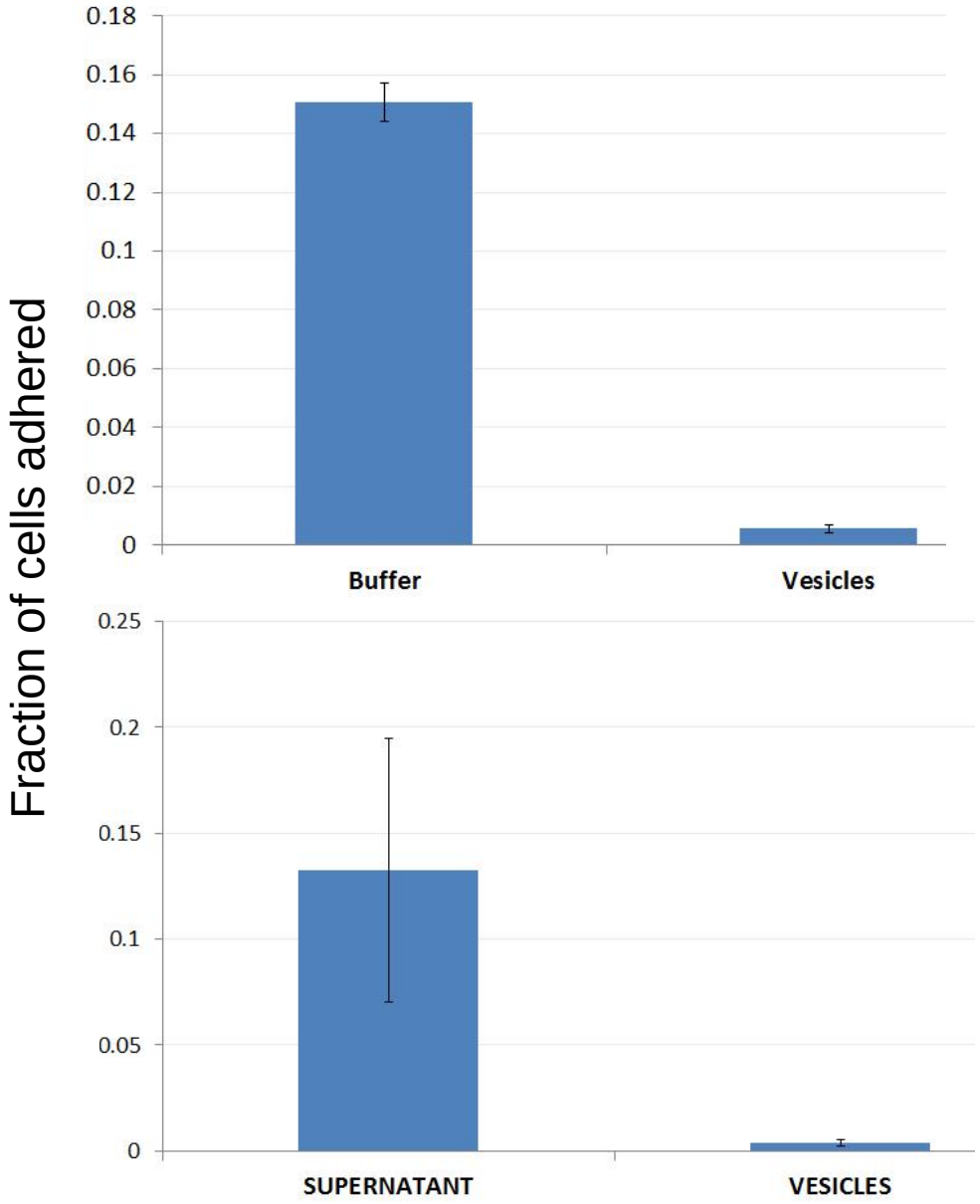


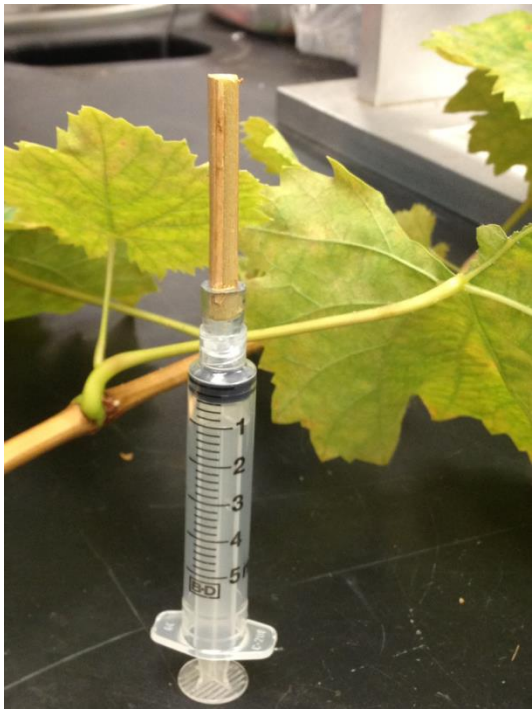
DSF production suppresses the release of vesicles by *Xylella fastidiosa*



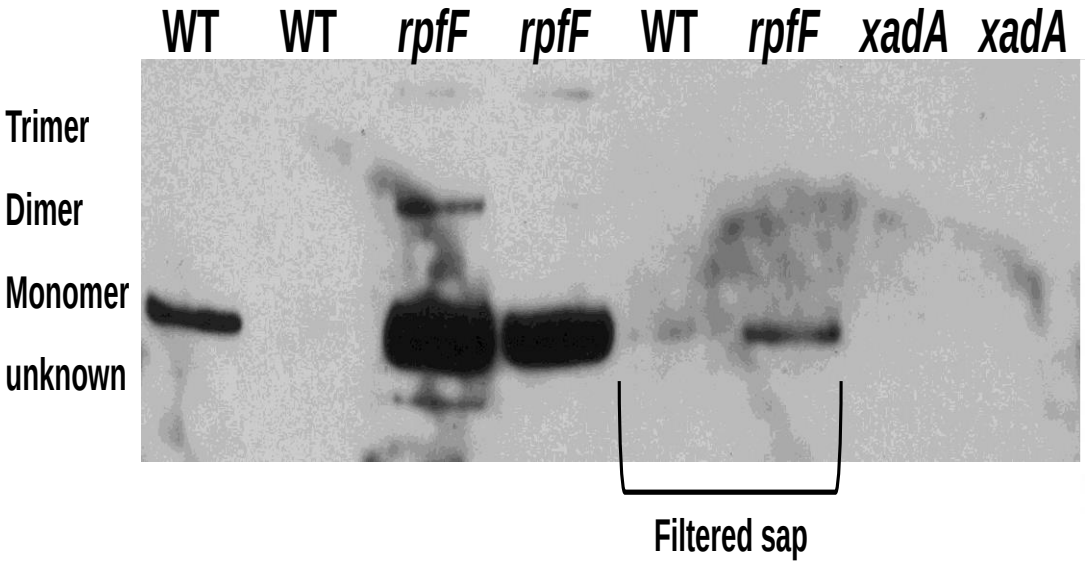


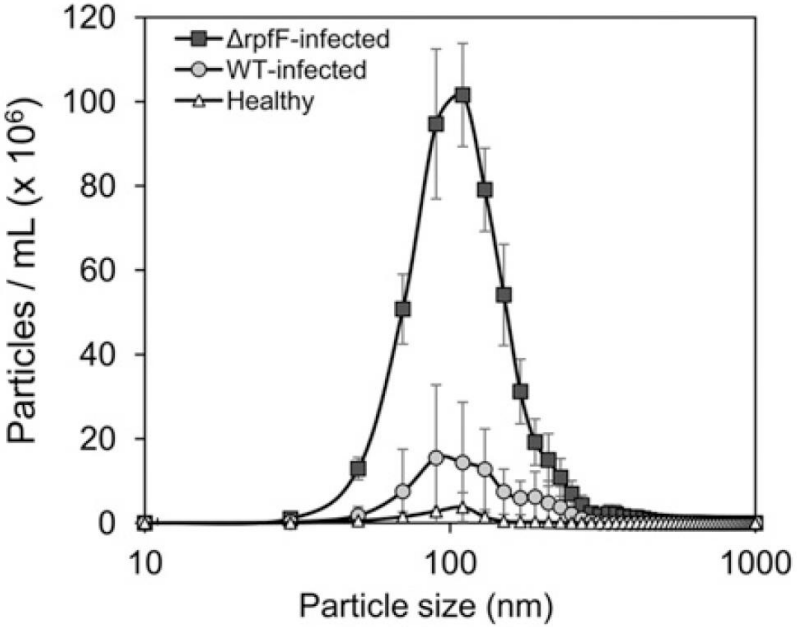
Xylella fastidiosa does not stick to xylem vessels in the presence of membrane vesicles





Membrane vesicles (XadA) abundant in xylem sap of infected grape





Disease control

<u>The goal</u>: Confuse pathogen by exposing it to excessive amounts of signal molecule even when it is in low population sizes

The expectation: The premature presence of DSF in vessels will suppress extracellular enzyme production and thus both movement and multiplication, as well as vesicle release, while increasing adhesiveness - thus also reducing movement

How?

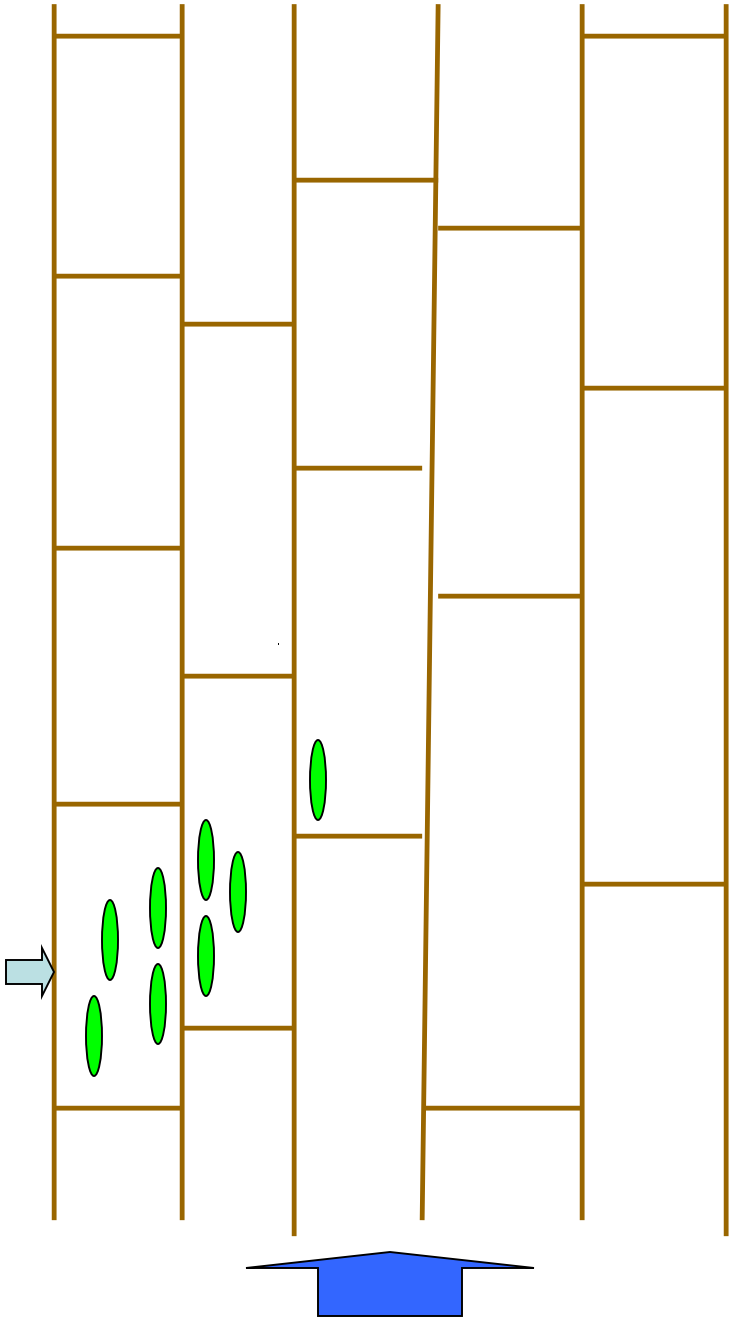
Transgenic DSF-producing plants
Direct application of DSF
Endophytes that produce DSF?

Early - Inoculum becomes mobile and starts to grow and spread

Non-mobile

Mobile

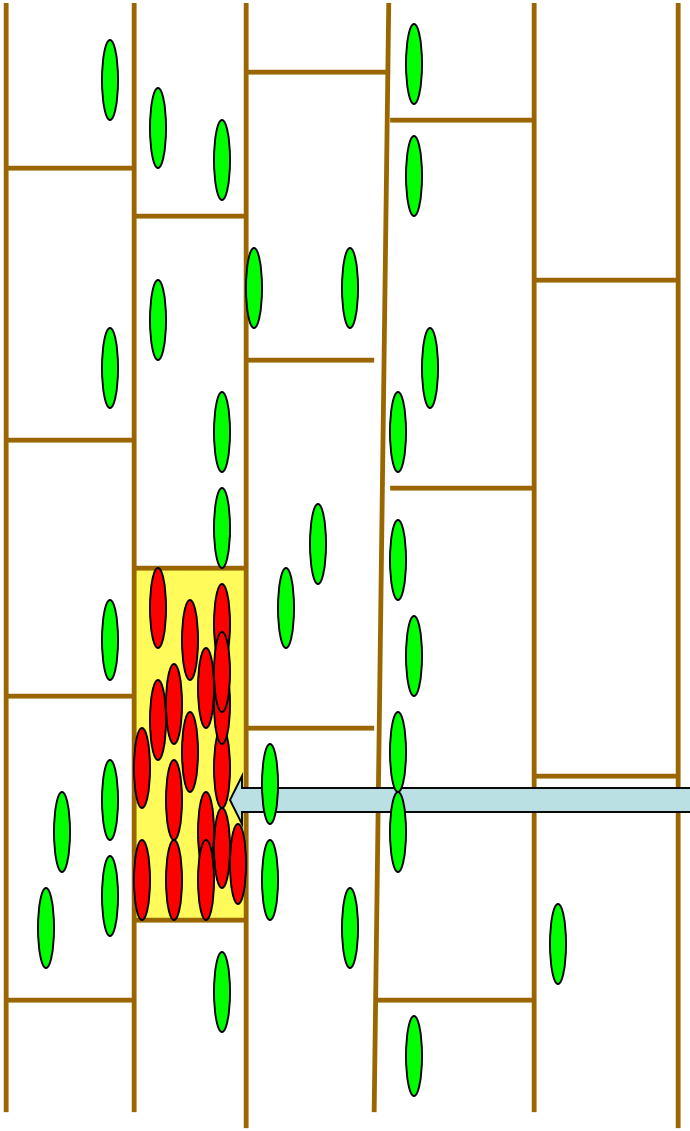
Sharpshooter Inoculation



Later- Extensive growth and spread of *X. fastidiosa* – some vessels become crowded and accumulate DSF – suppression of further growth and movement in that vessel – acquired by insects

Non-mobile

Mobile



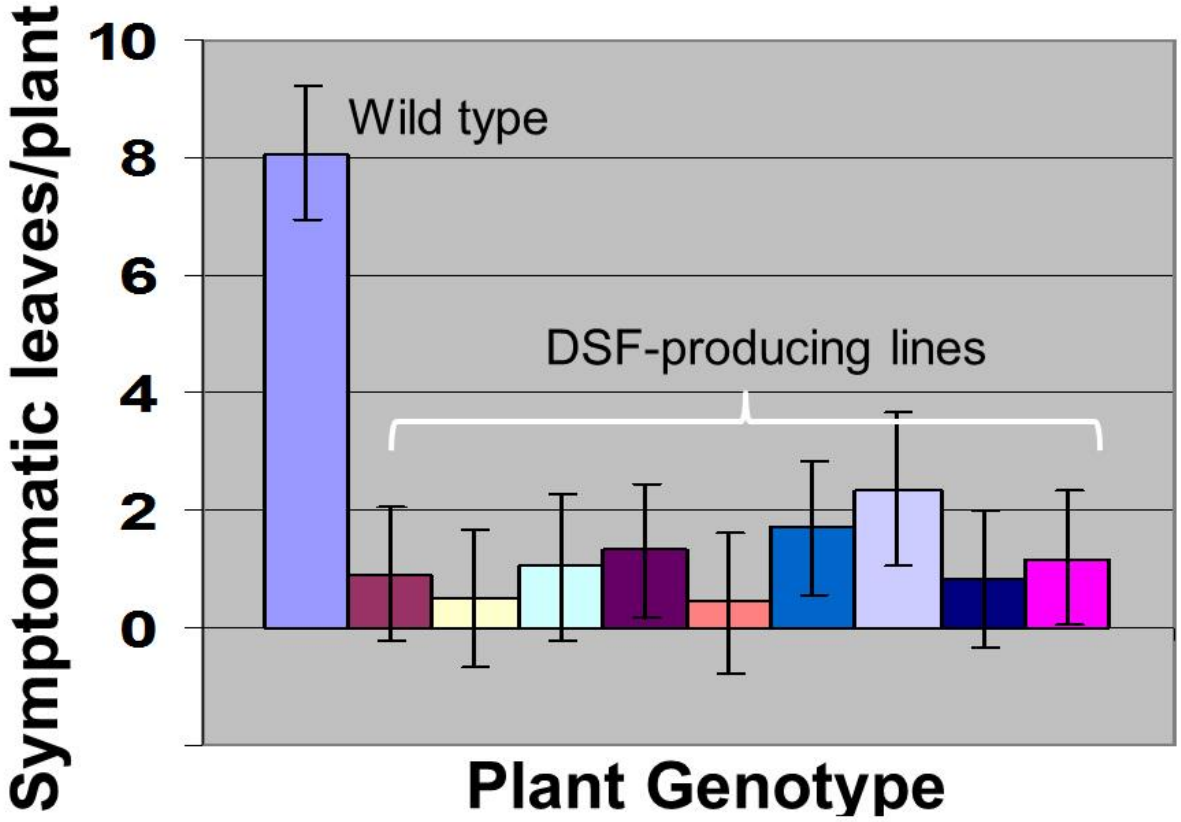
Sharpshooter Acquisition

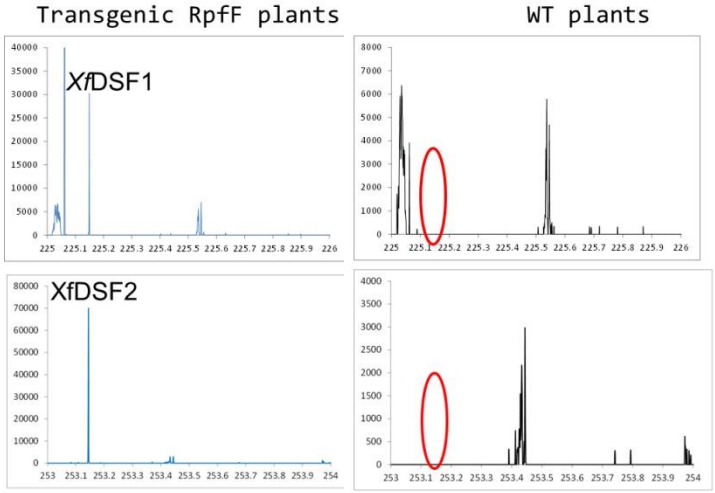
In transgenic DSF-producing plant signal would be high at all times, restricting growth and movement

Non-mobile

Sharpshooter Inoculation

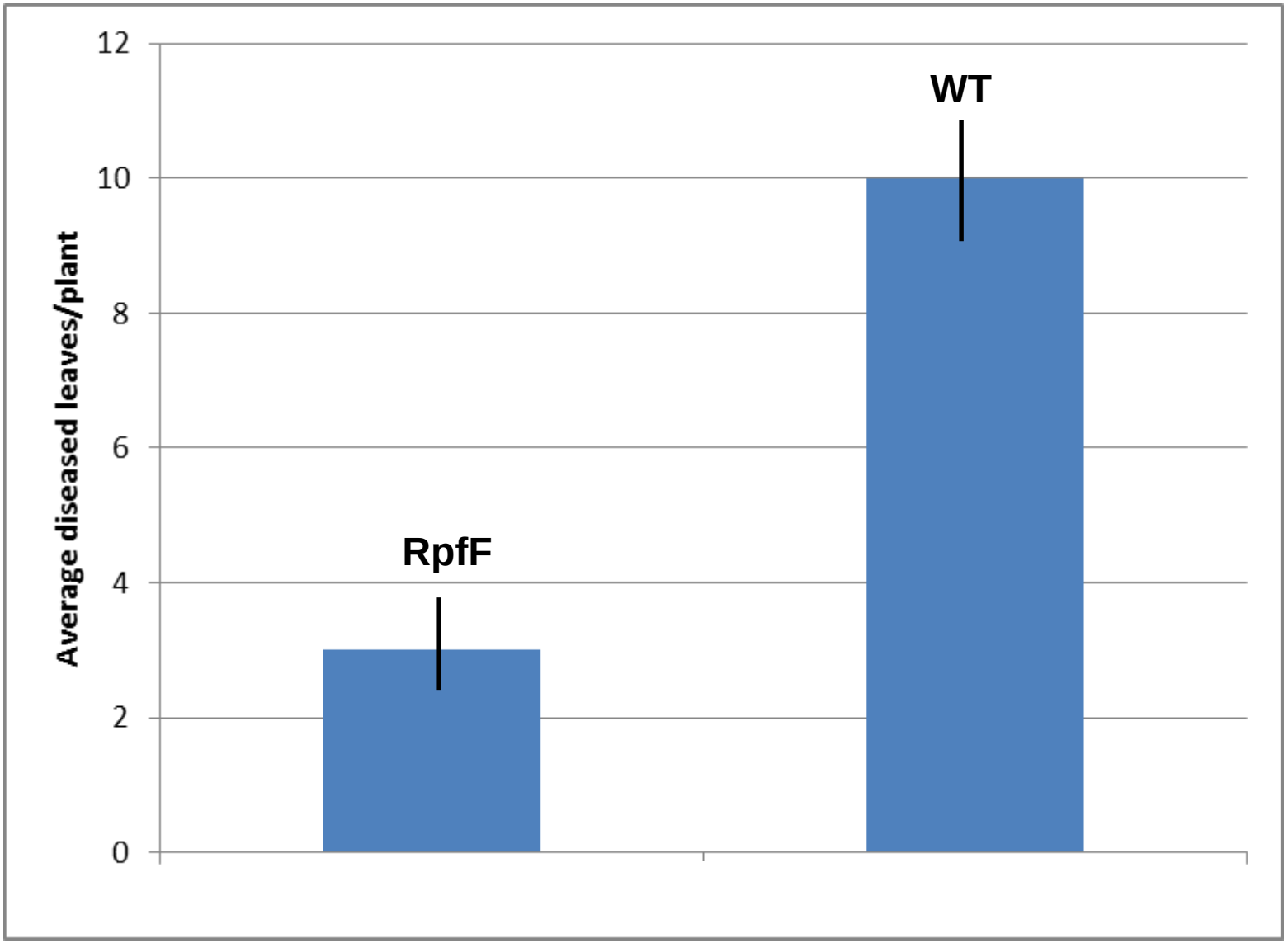
Grape transformed with *rpfF* gene from *Xylella fastidiosa* that produce DSF are <u>much</u> more resistant to Pierce's Disease compared to wild-type grape





Field trials of transgenic grapes conducted at UC Davis and Riverside

Severity of PD much lower on RpfF-expressing Freedom – Solano County August, 2012



Large reduction in disease in DSF-producing plants - Riverside County October, 2012

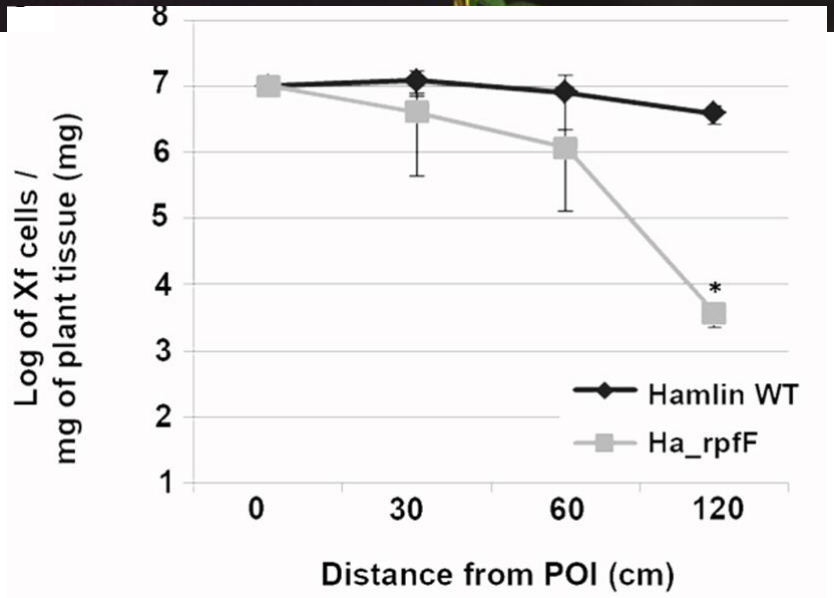
DSF-producing

Normal grape

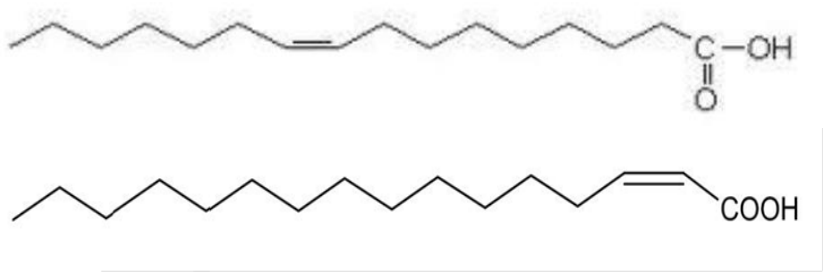
Expression of *Xylella fastidiosa rpfF* in Citrus reduces growth and movement of pathogen and Citrus Variegated Chlorosis symptoms

Hamlin WT

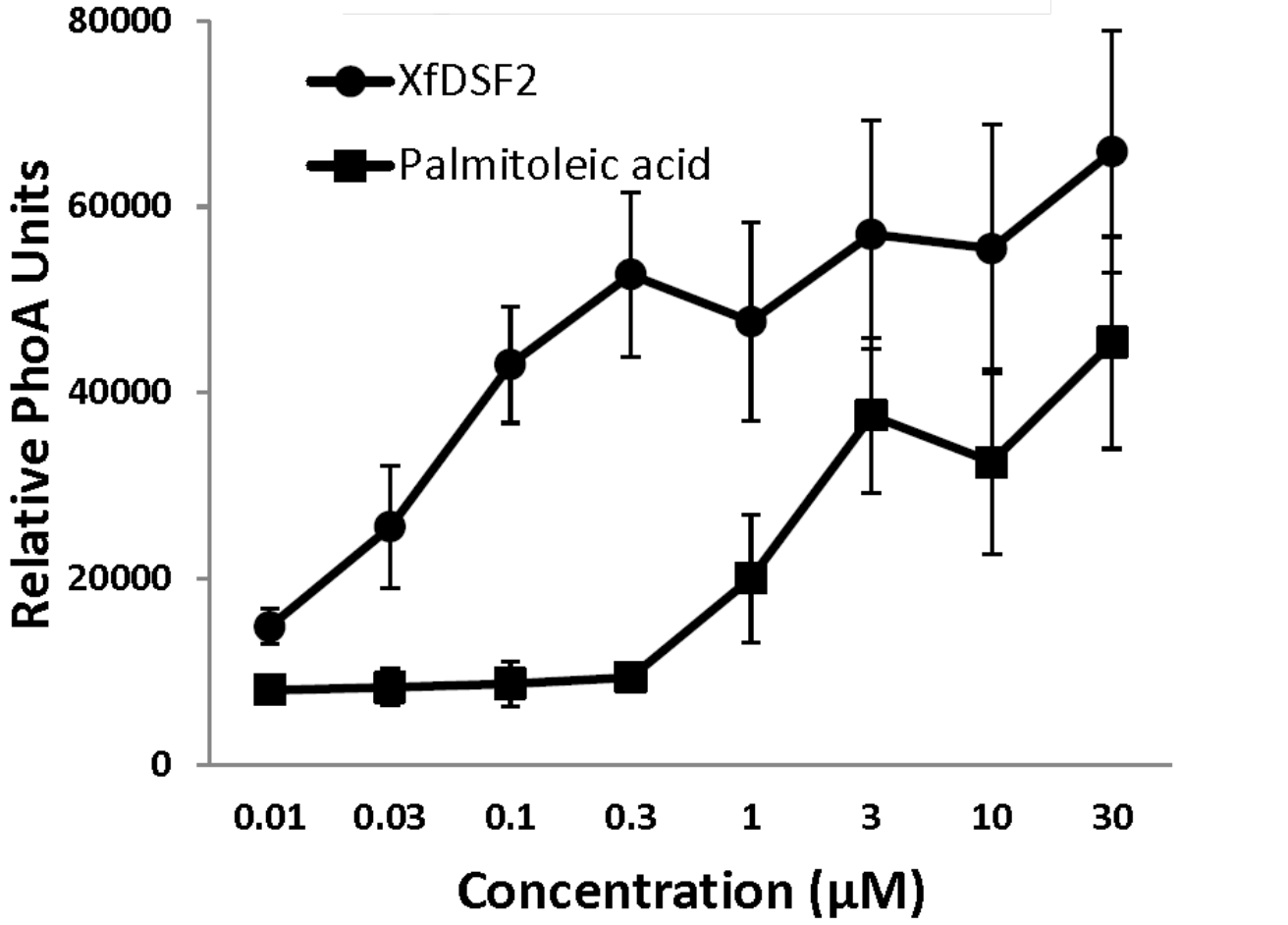
Hamlin rpfF



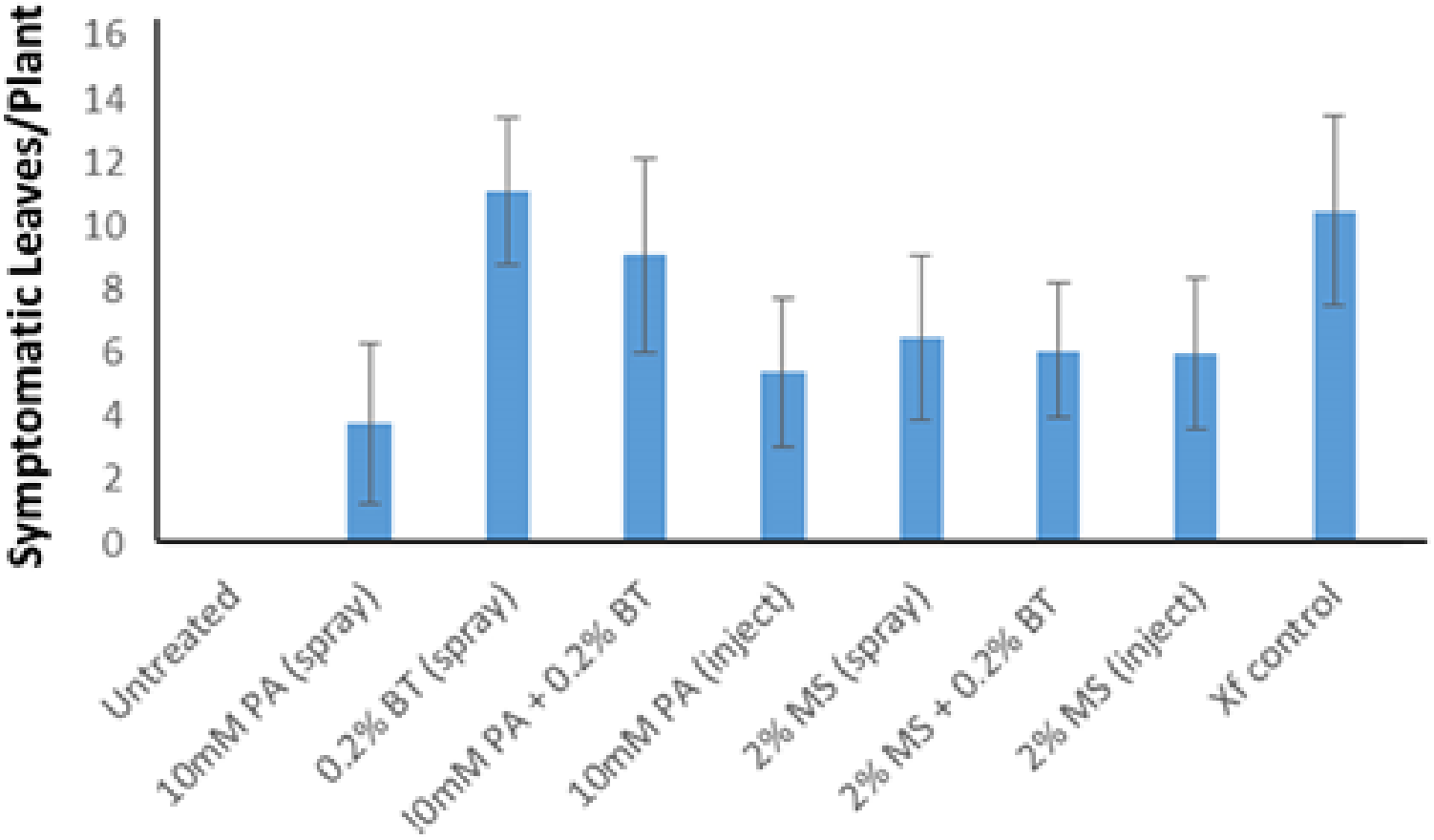
Palmitoleic acid is attractive as a commercially available signal for *X. fastidiosa*



XfDSF2

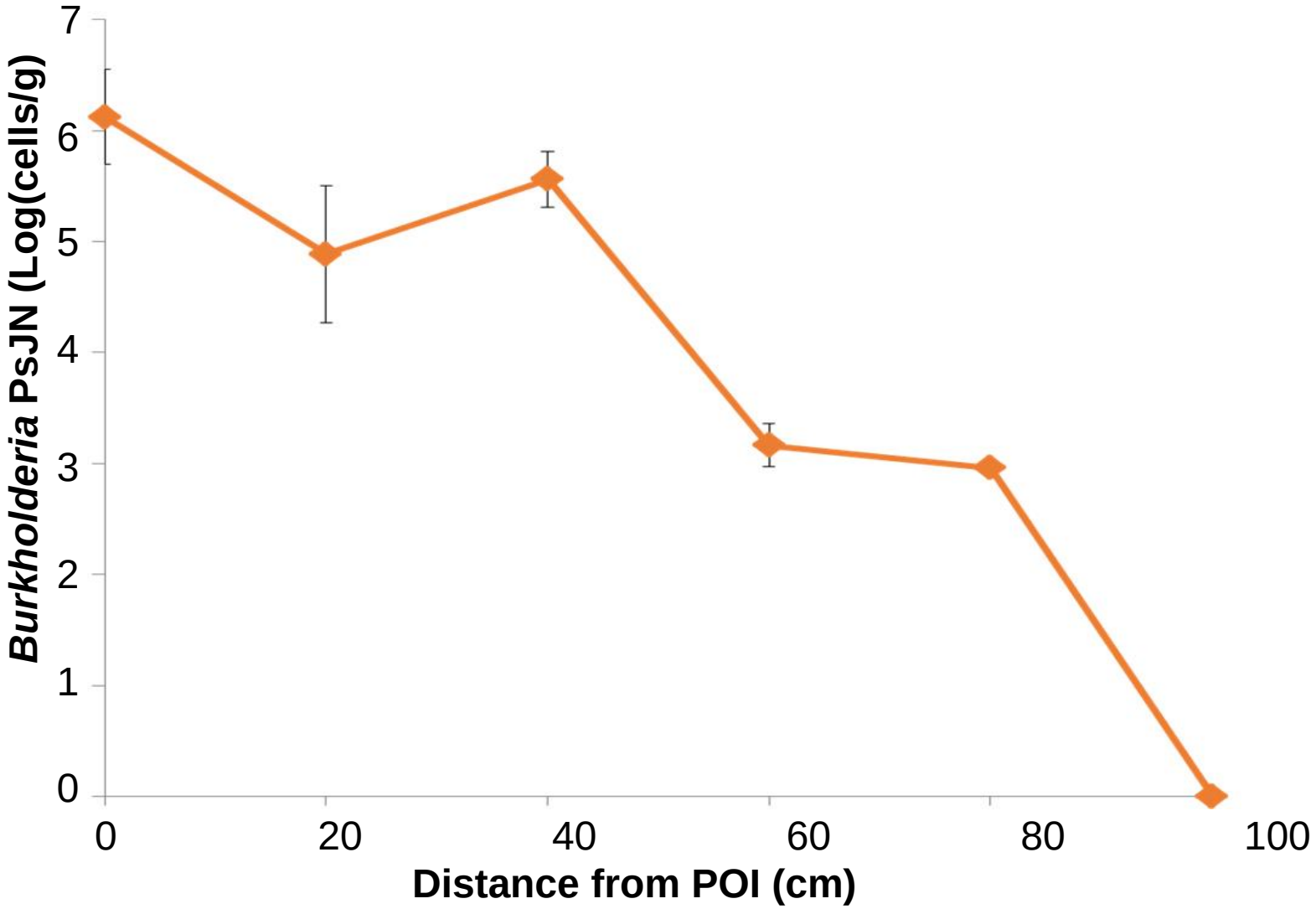


Some disease control can be conferred by topically applied signaling molecules

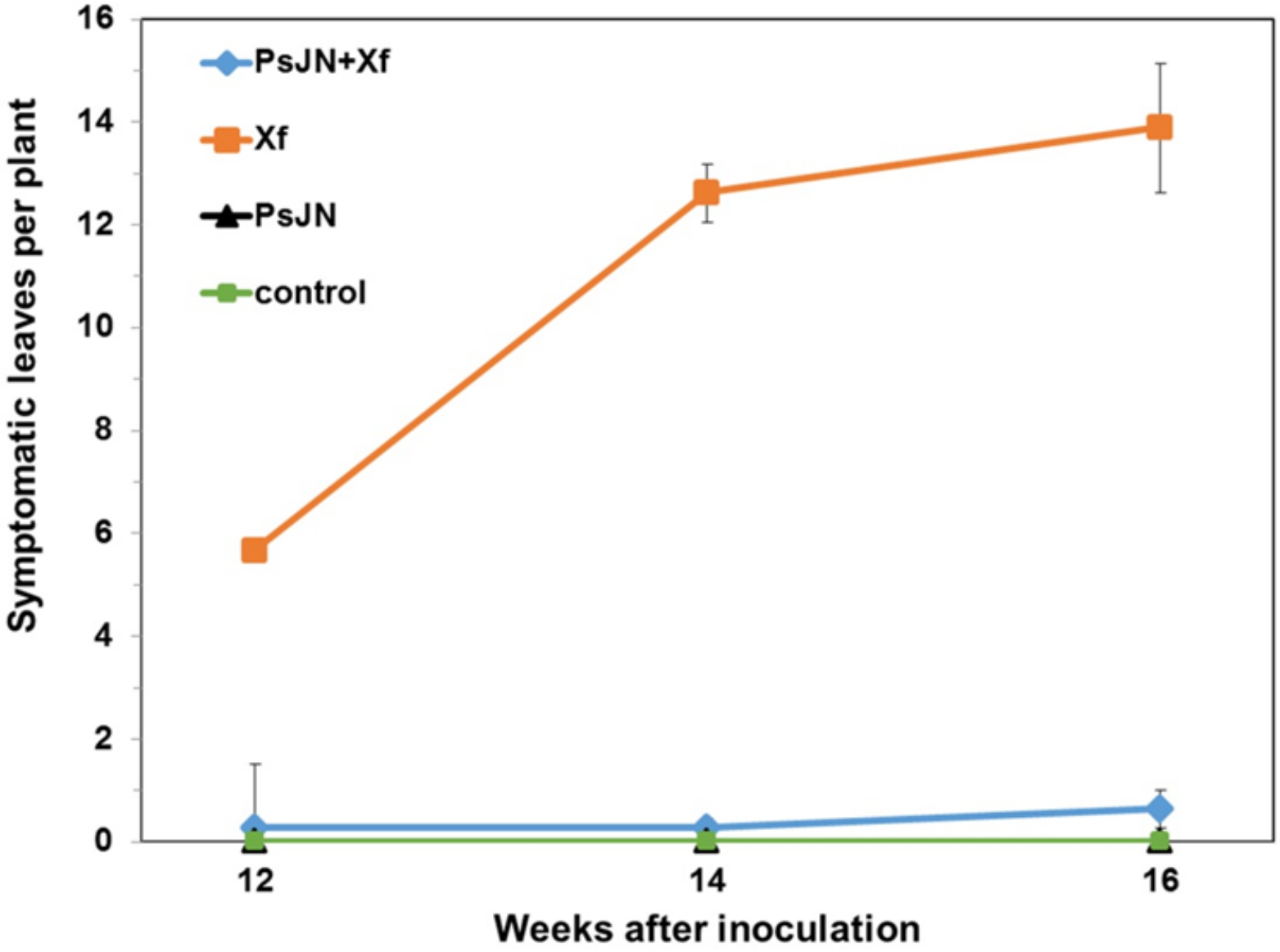


Biological Control of Disease

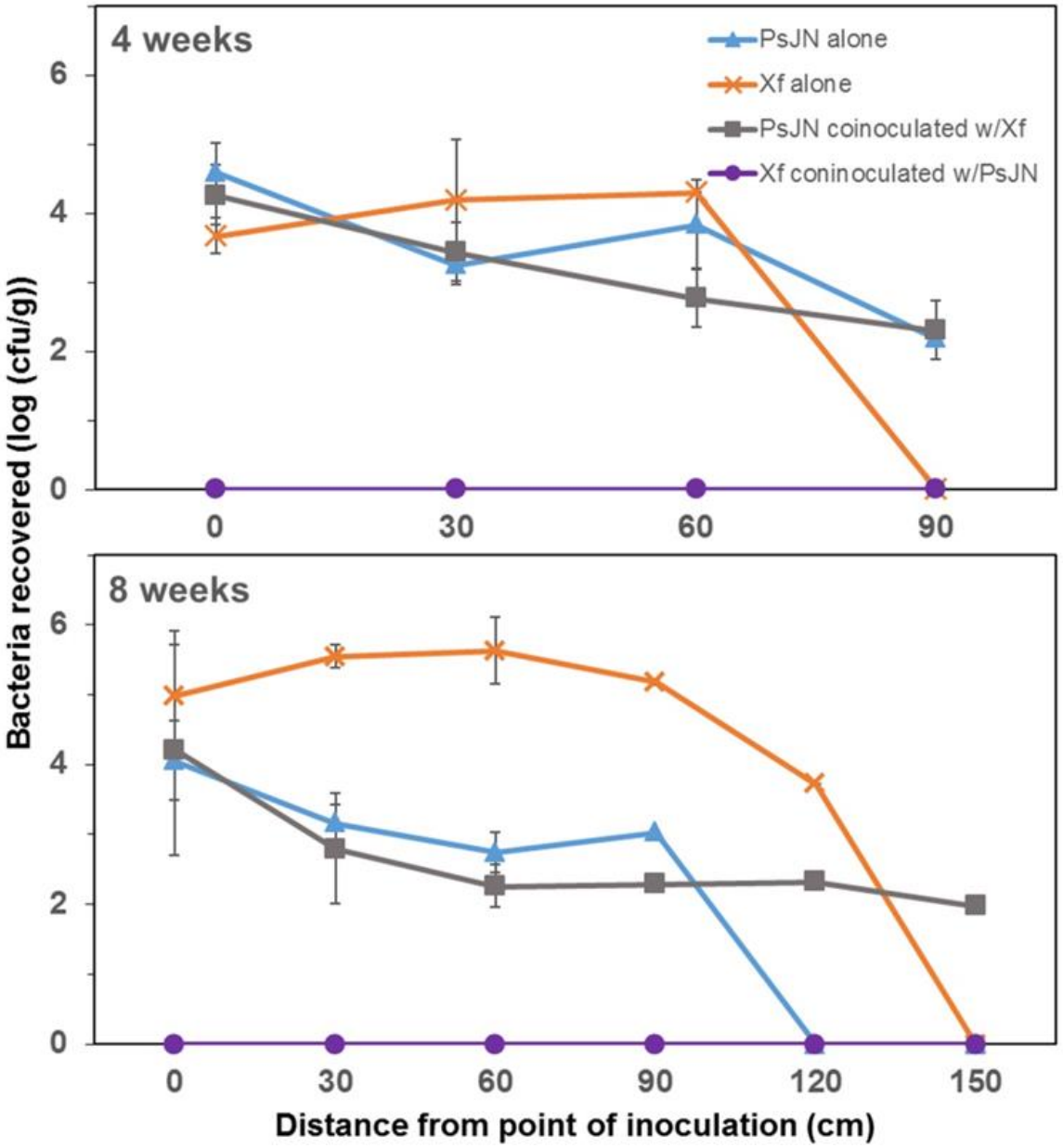
Paraburkholderia phytofirmans colonizes grape xylem, unlike other bacteria tested



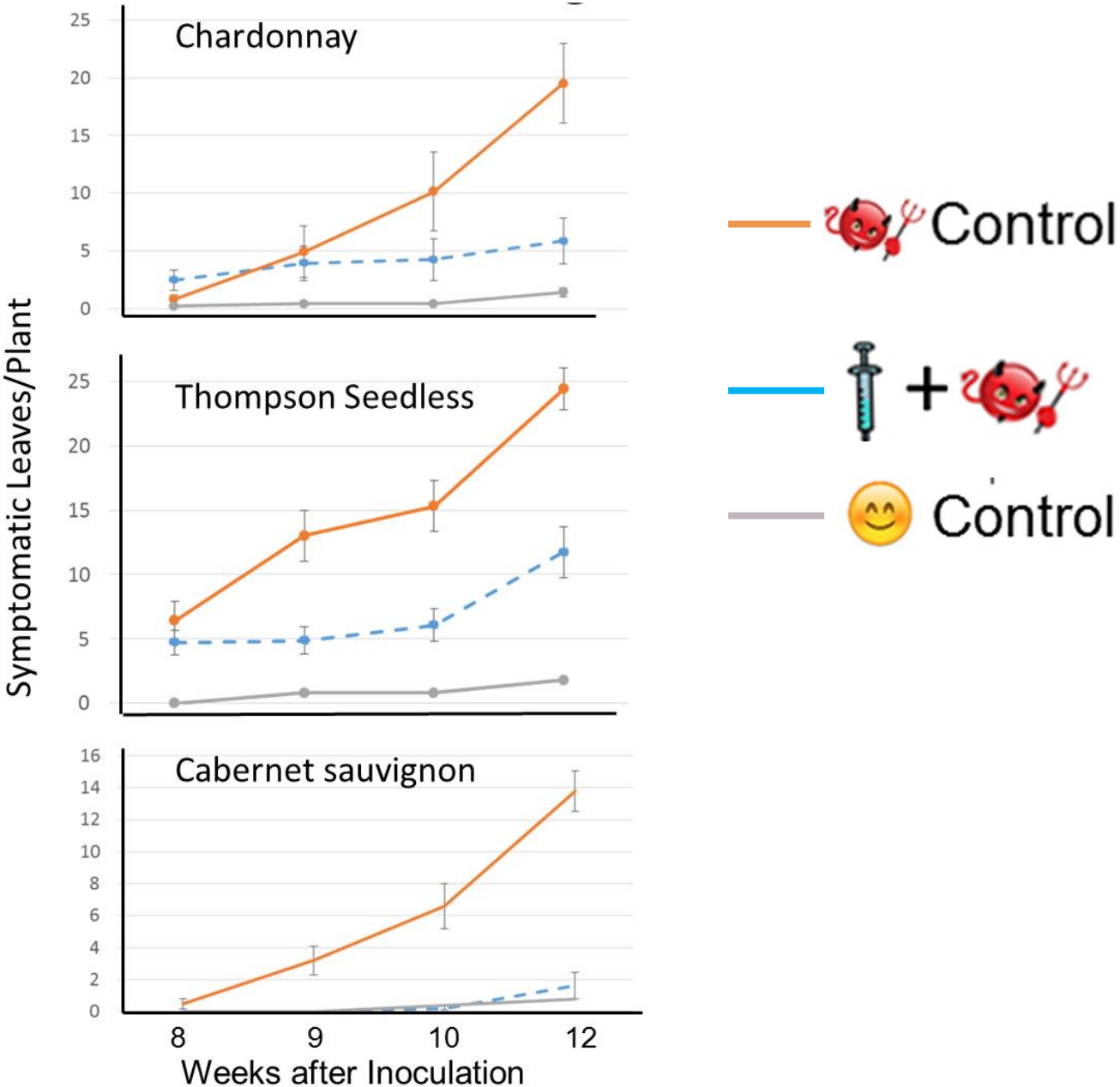
Inoculation of Parab*urkholderia phytofirmans itself* with *Xylella fastidiosa* greatly reduces disease severity



Co-inoculation of Xf and *Paraburkholderia* results in HUGE reductions in the population size of the pathogen in plants

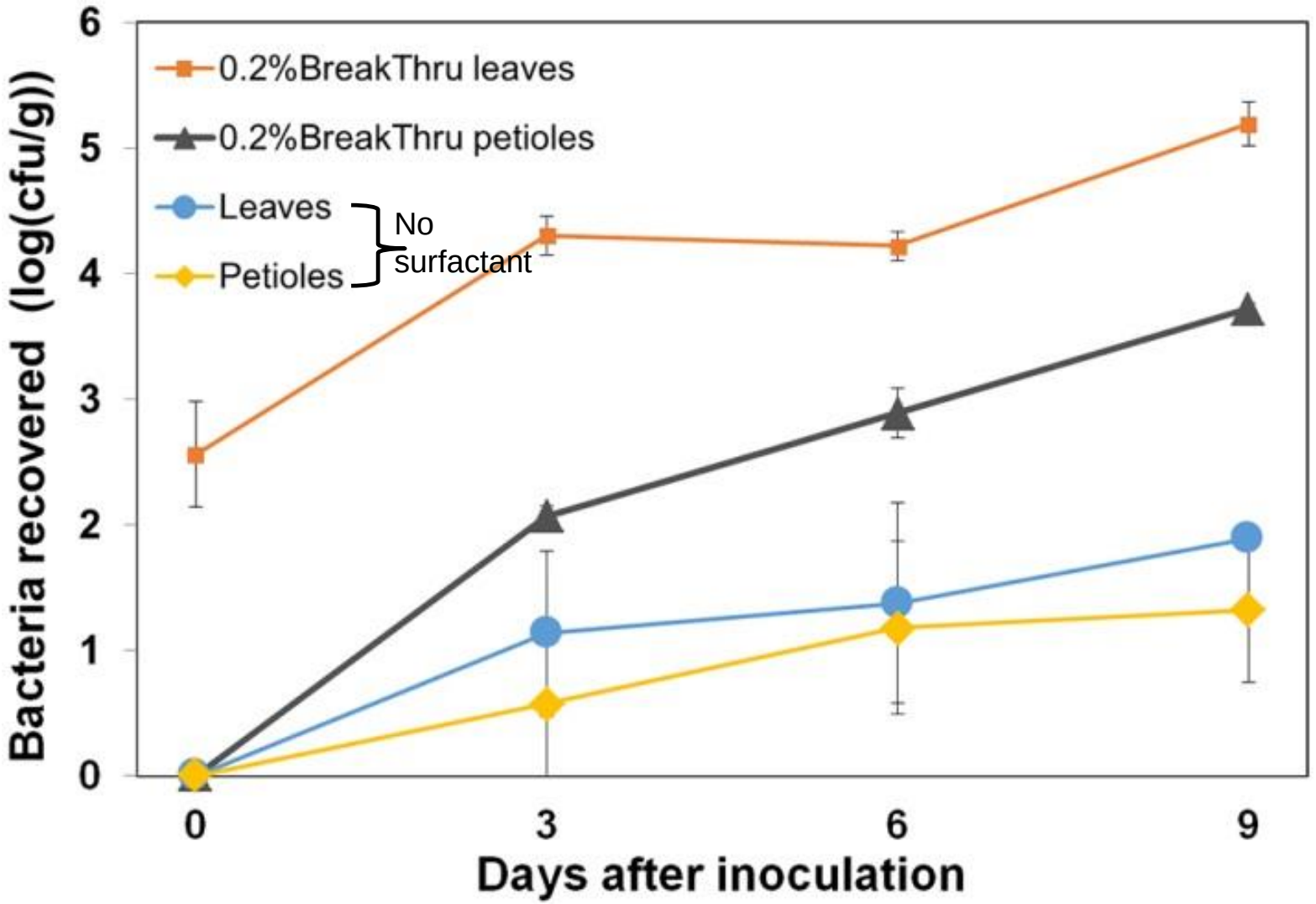


Inoculation of *Paraburkholderia* at time of *X. fastidiosa* inoculation reduces disease in all grape varieties tested

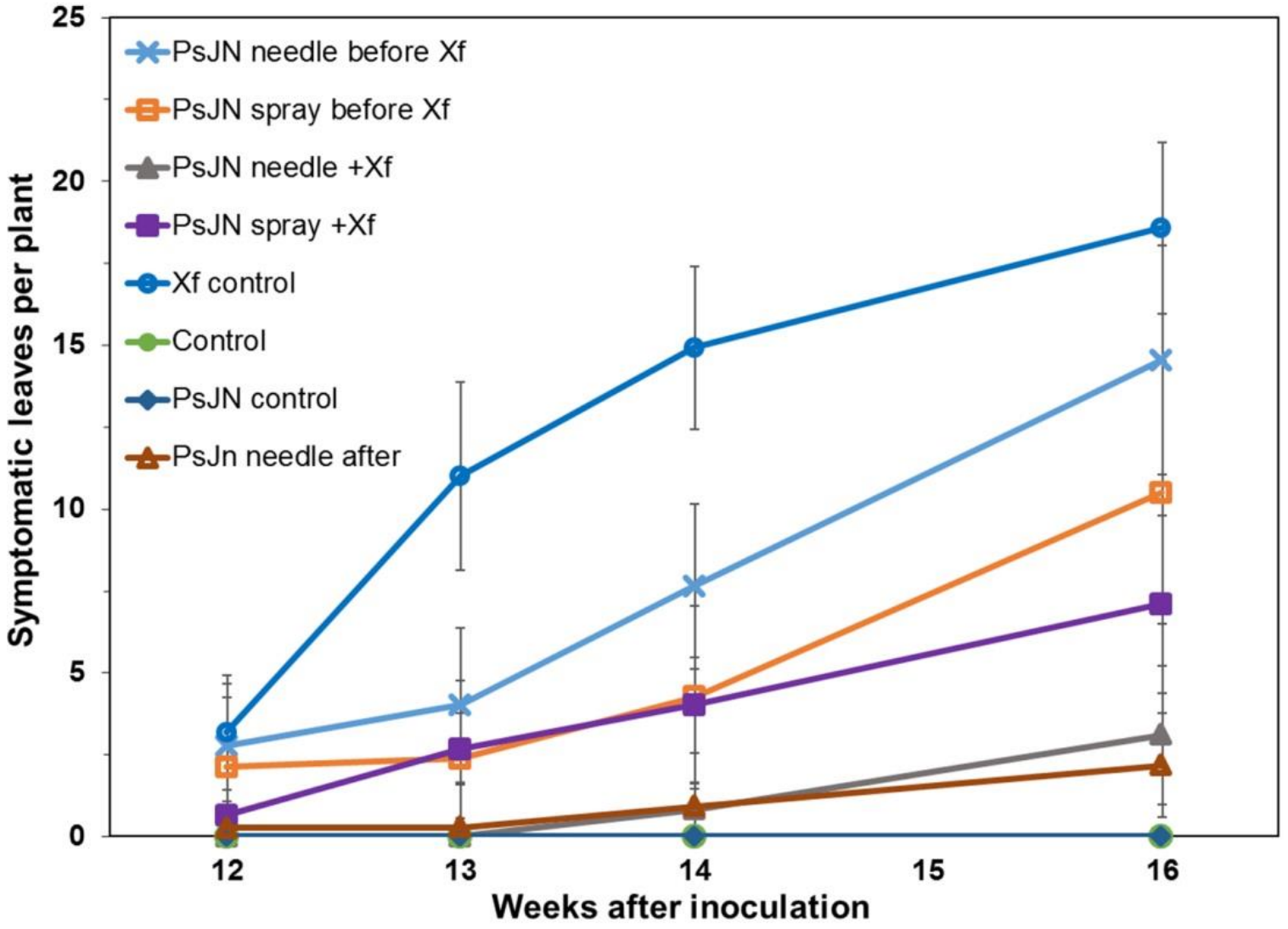


Bacteria can be introduced into plant tissue with penetrating surfactants

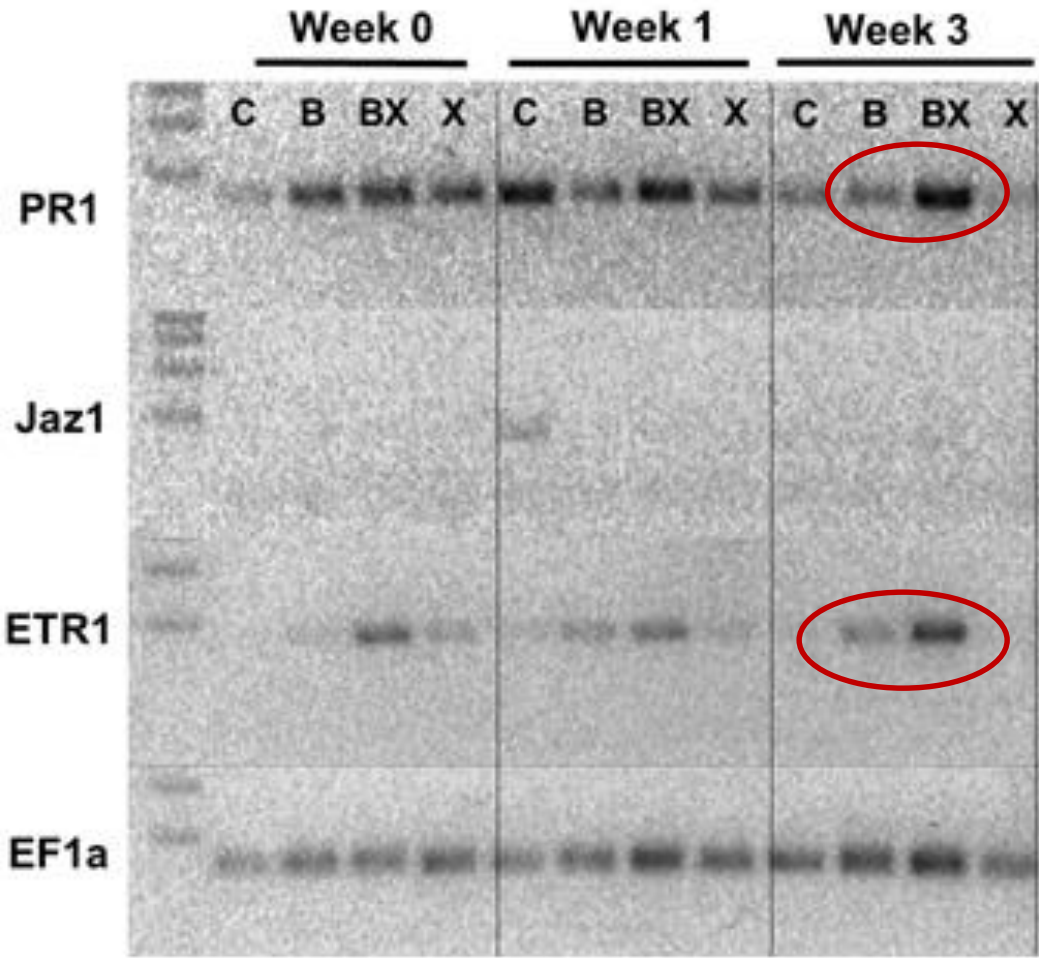
Large populations of *Xylella fastidiosa* within leaves immediately after spraying



Paraburkholderia phytofirmans mediates disease control even after infection of grape with *Xylella fastidiosa* when applied in different ways

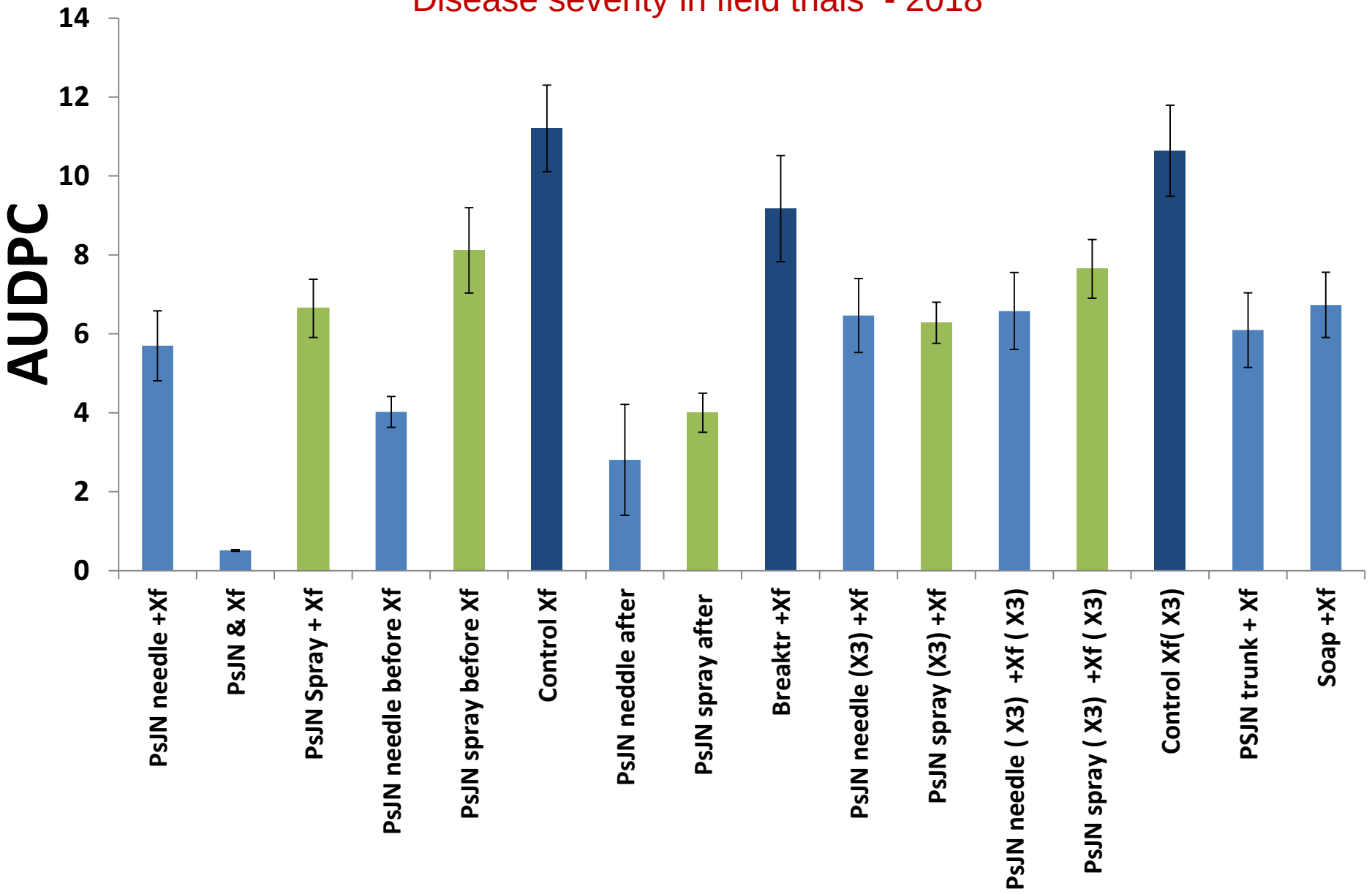


Priming of innate immune system of grape by *Paraburkholderia phytofirmans*

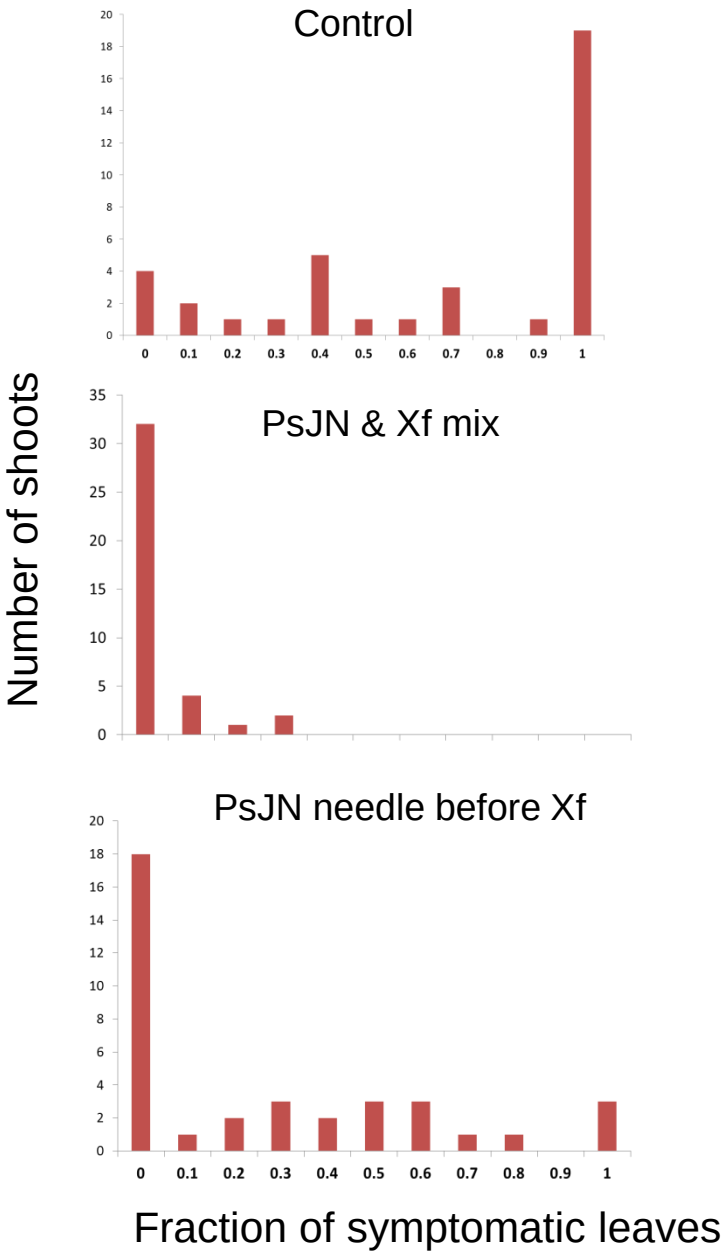


B = *Paraburkholderia* alone
BX = *Paraburkholderia* + *Xylella*
X = *Xylella* alone

Disease severity in field trials - 2018

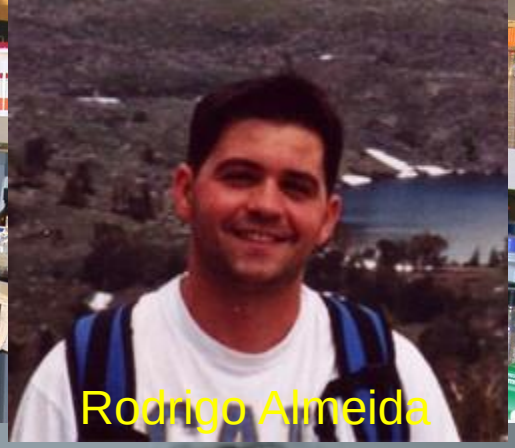


Disease reduction conferred by PsJN was associated with complete control on most shoots rather than reduced severity on a given shoot – All or none response



Nian Wang

Aline da Silva



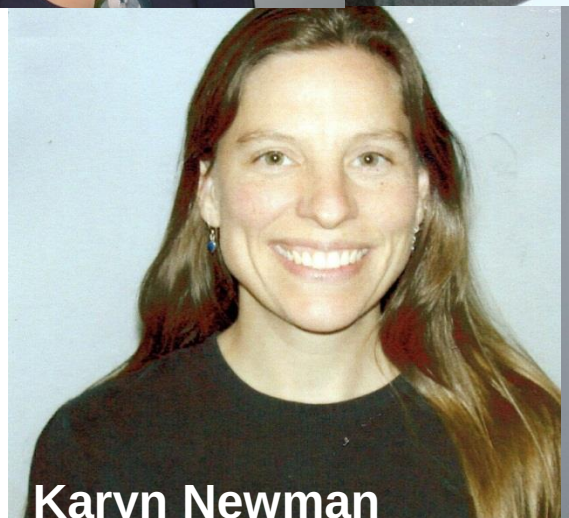
Rodrigo Almeida

Kenji Yakota

Elena Antonova

Subhadeep Chatterjee

Clelia Baccari



Karyn Newman

Ellen Beaulieu

Miki Ionescu