PSA in the Apoplasm

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Te Whare Wānanga o Waikato

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Apoplasm – the space outside the plant cell membrane

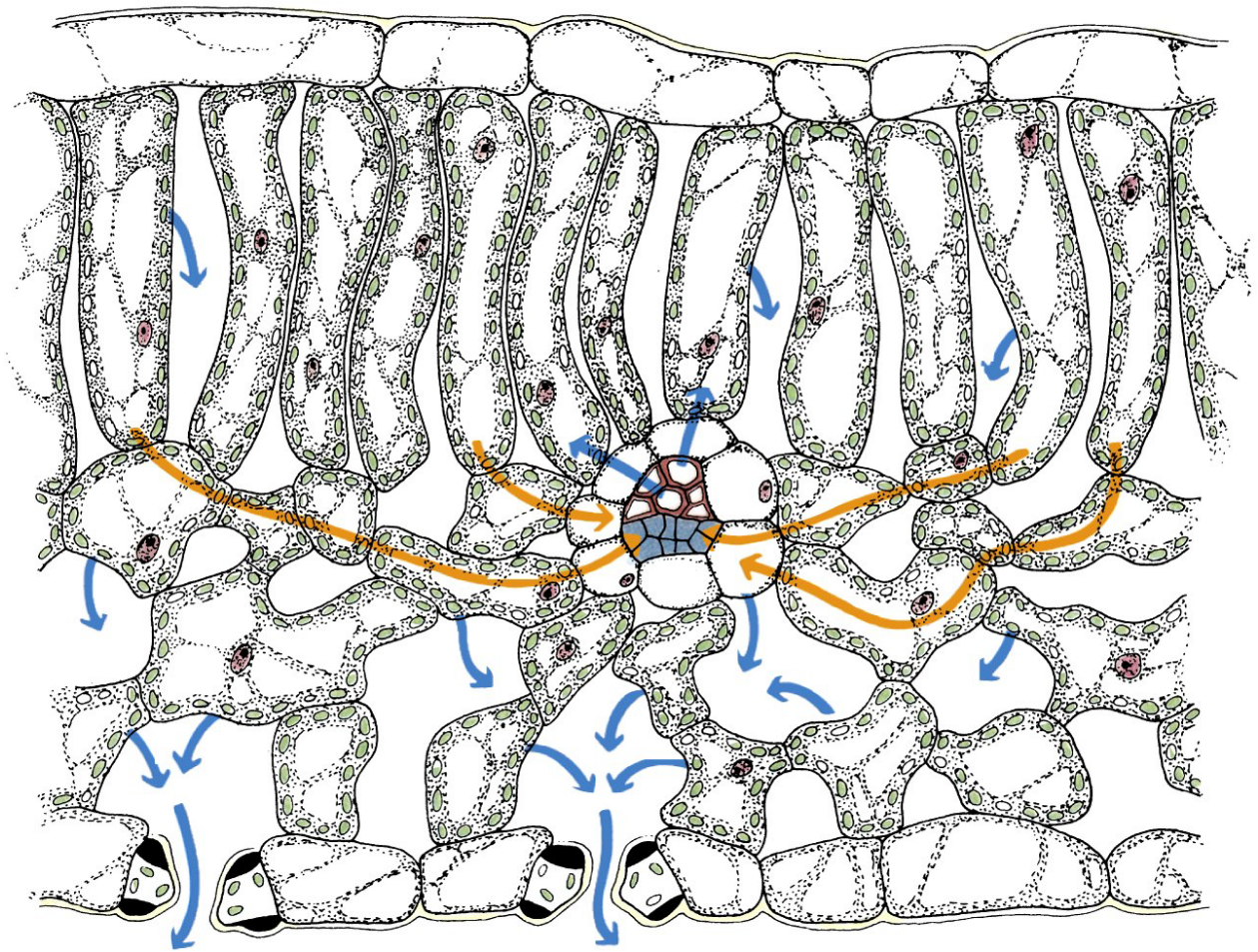
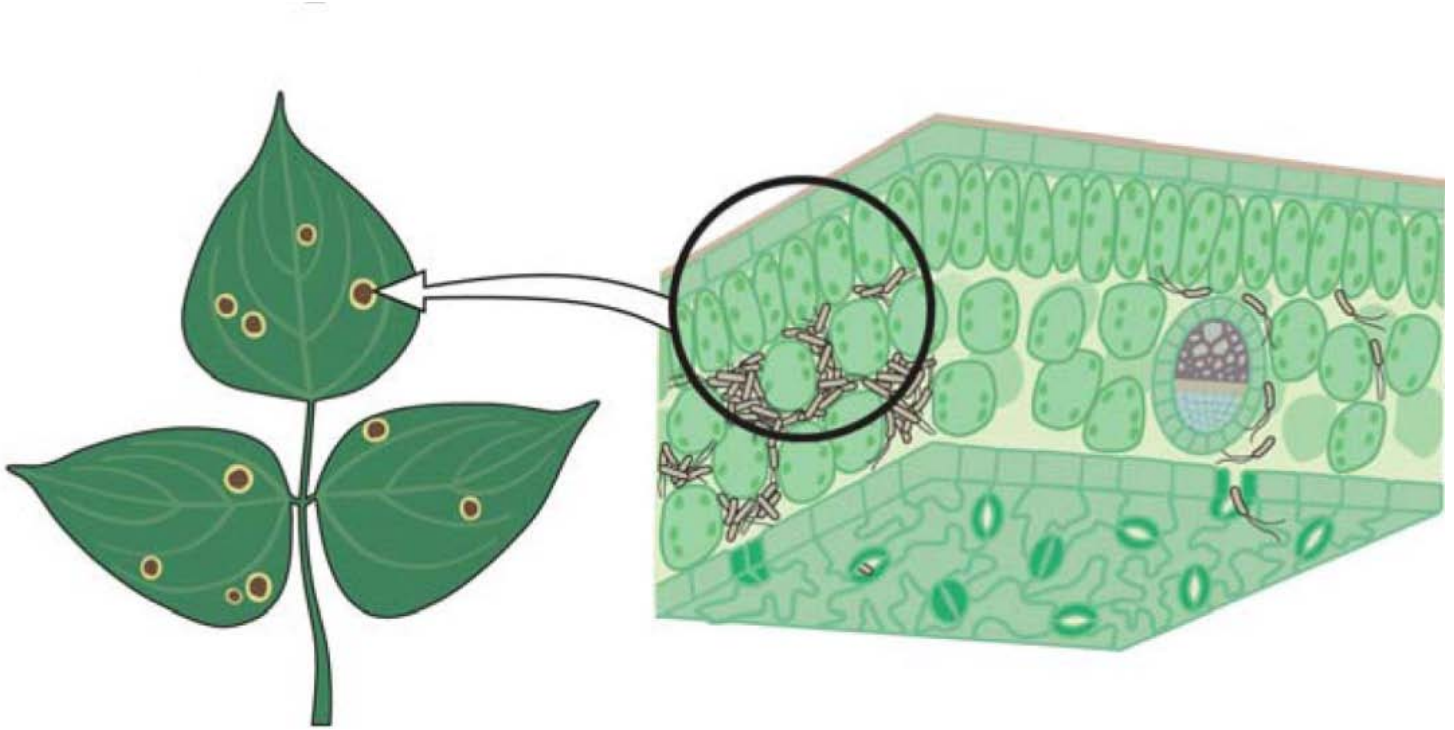


Figure 30-21
Biology of Plants, Seventh Edition

PSA starts by colonizing the apoplasm

- Enters through wounds or stomata
- Modifies the apoplasmic sap to feed, reproduce and grow
- May move through the apoplasmic space to the vascular tissue, or back onto the plant surface



Melotto *et al. Annu Rev Phytopathol*. 2008 ; 46: 101–122

Outline

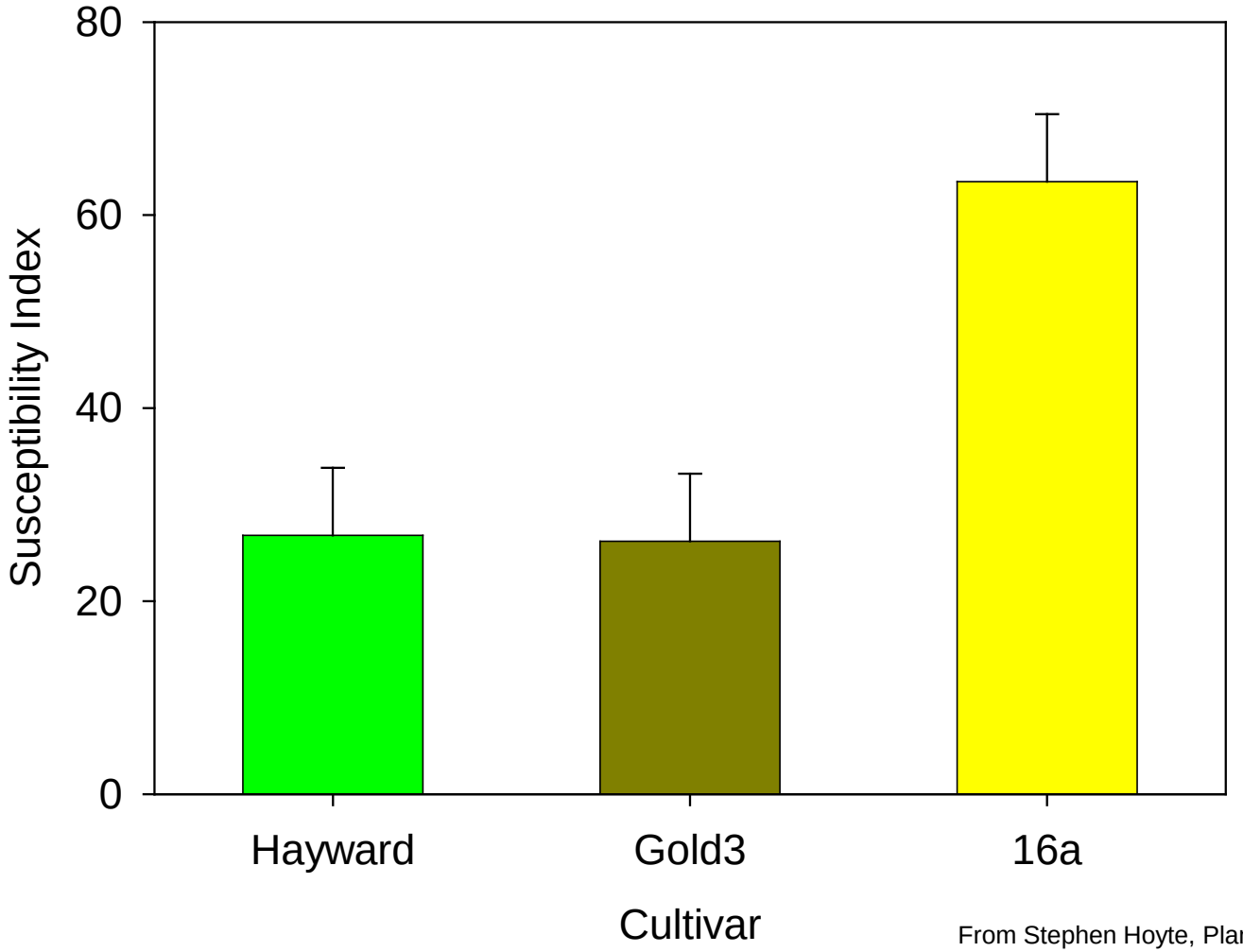
PSA in the Apoplasm

- **Proliferation in the apoplasm**
- Entry via leaf scars
- Movement within the xylem
- Impact on xylem hydraulic functioning
- Collection and analysis of xylem sap

Proliferation in the apoplasm

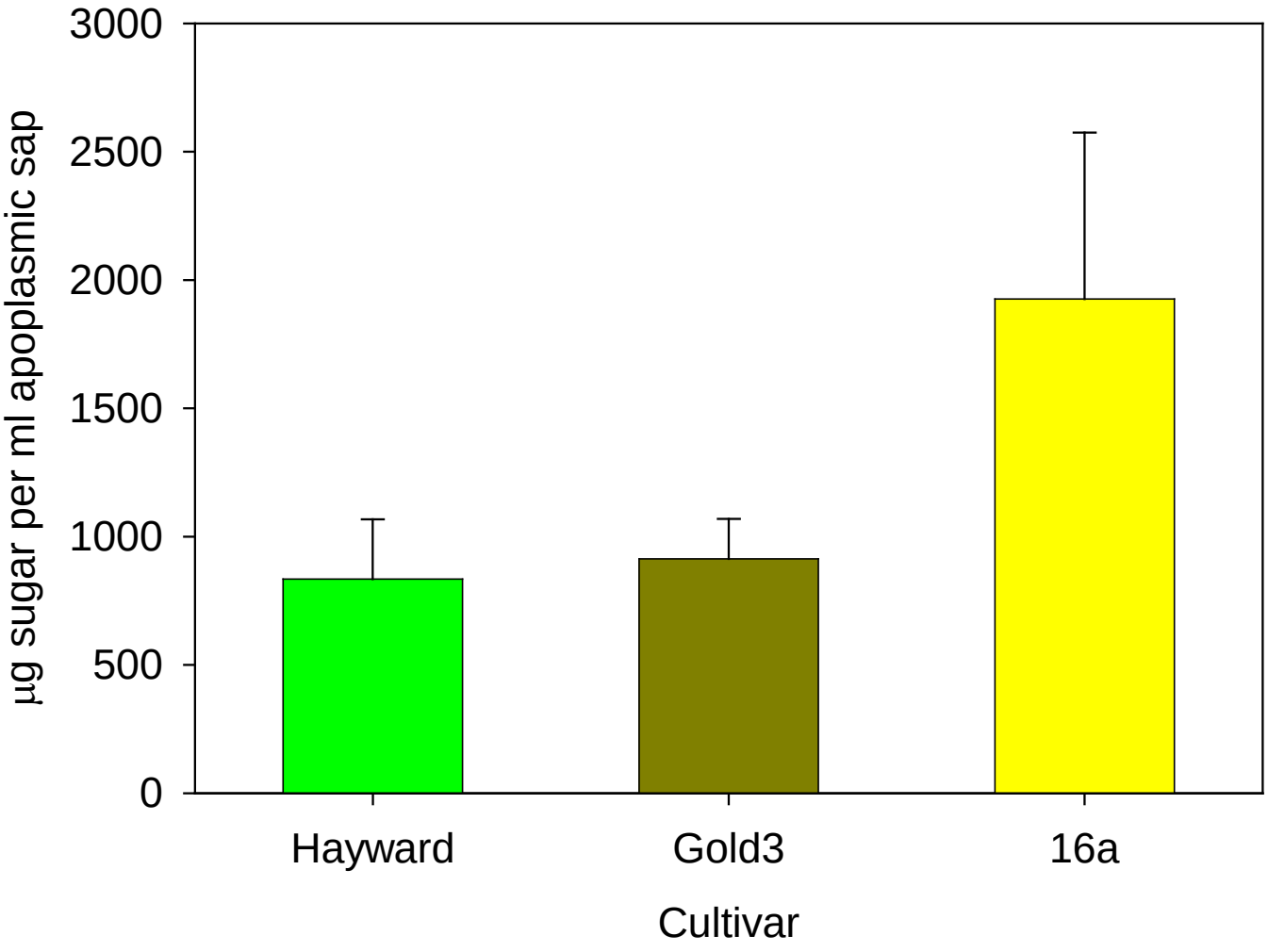
- Is variation in susceptibility between cultivars related to the chemistry of the apoplasmic space?
- Wash out technique developed to extract apoplasmic sap
- Sap composition analysed, tested as a culture medium

Faster stem lesion growth in Hort16A …



From Stephen Hoyte, Plant and Food Research
Woody stem bioassay index
(5 replicates/bioassay/dose rate)
High and Low inoculum doses combined
Mean of two bioassays

Is correlated with more sugars in the apoplasm



Proliferation in the apoplasm

Conclusion:

- Cultivars vary in the chemistry of the apoplasmic space, sugar concentrations are higher in the more susceptible cultivar

Next steps:

- More robust analysis of apoplasmic chemistry
- Sample apoplasmic sap regularly throughout the year
- Examine bacterial growth and metabolism on apoplasmic sap
- Investigate bacterial ability to manipulate sap composition

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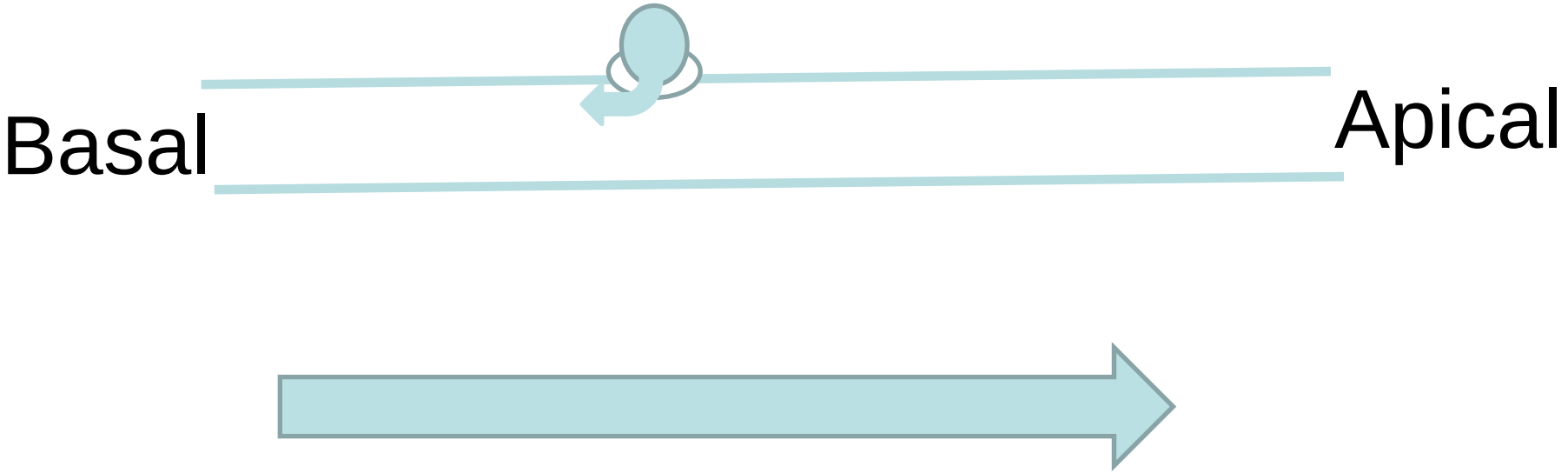
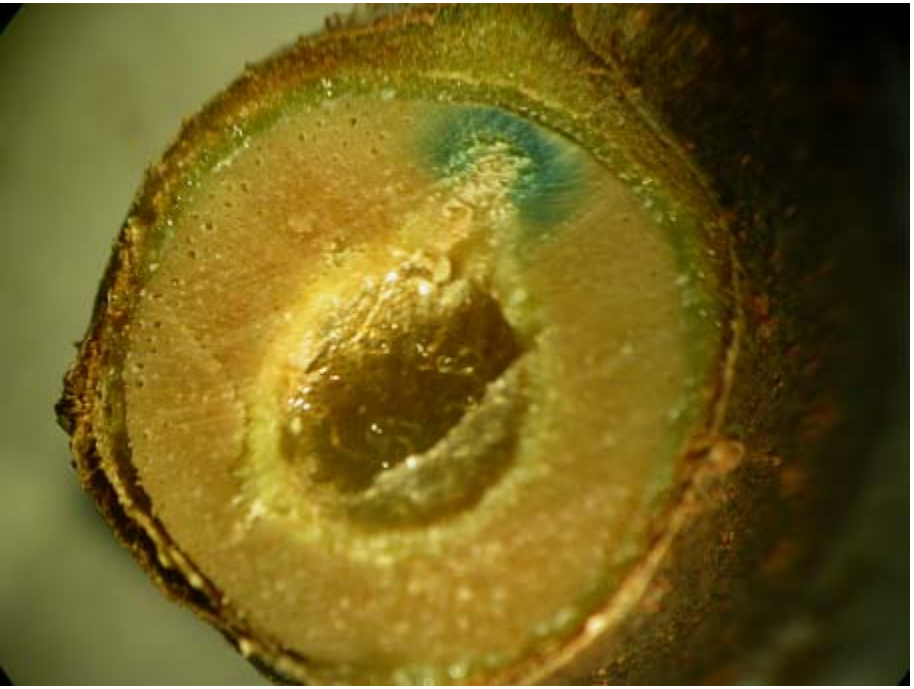
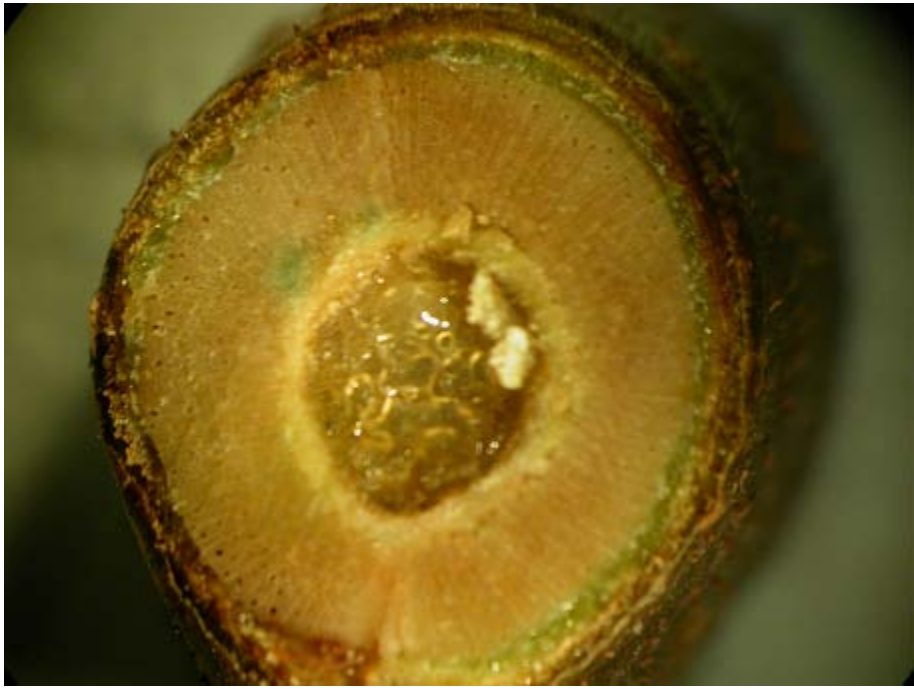
Can PSA infect through leaf abscission scars?



Fresh abscission scars permit water (and dye) flow into the shoot





PSA can be sucked through a proportion of leaf scars

Time from leaf fall (hours)	Proportion plates with PSA	% plates with PSA
2	3/25	12%
24	0/12	0%
48	1/10	10%

- PSA inoculum placed on leaf scar, suction applied to xylem at base of stem, solution collected and cultured
- Ability to pass through stem from scar suggests there are some open xylem vessels between the scar and the cut end of the stem
- Frequency of entry via leaf scar xylem should decline with time – more repetitions needed

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From leaf spots to shoot collapse

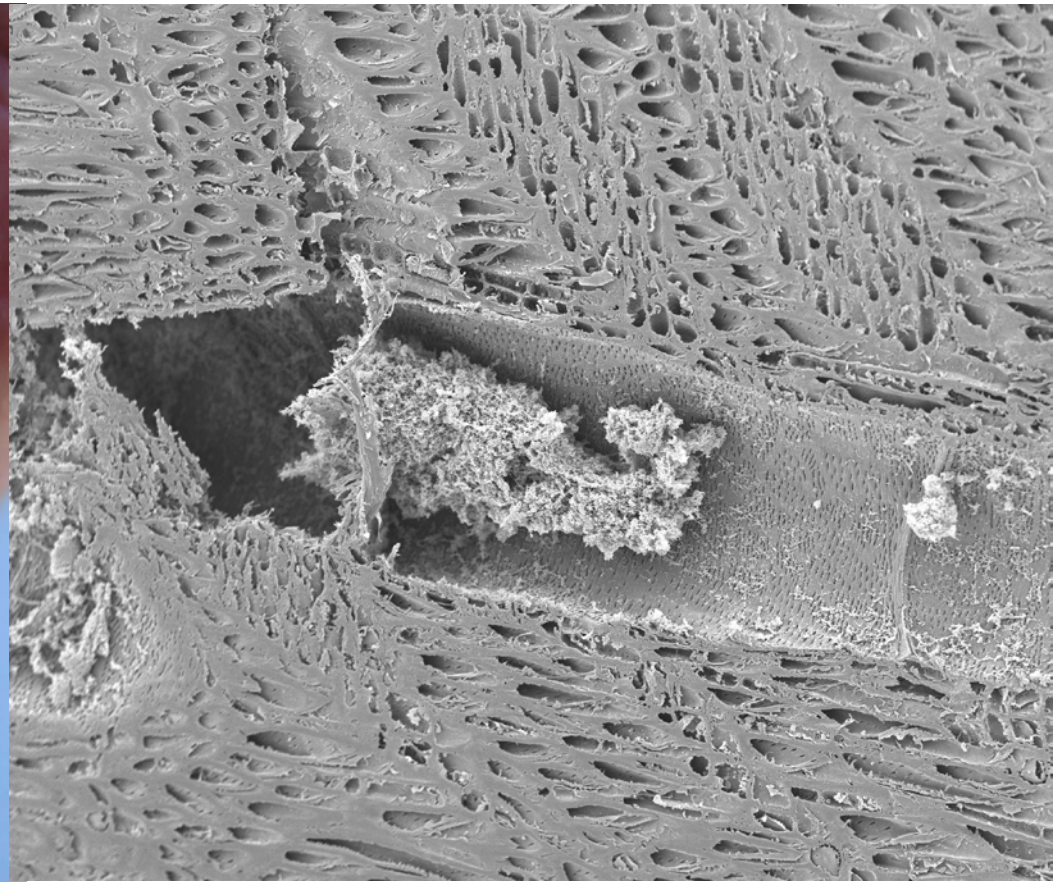
Matt Templeton, Plant and Food Research

Systemic symptoms – Cankers and Red Exudate

Bob Fullerton, PFR

Shane Max, Zespri

Movement within the xylem

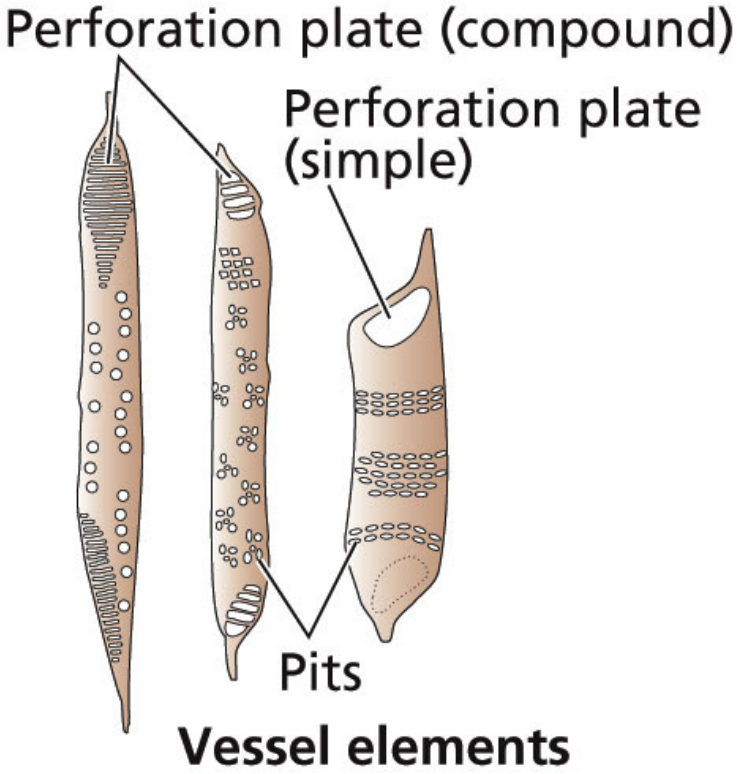


Mike Manning, Paul Sutherland, Plant and Food Research

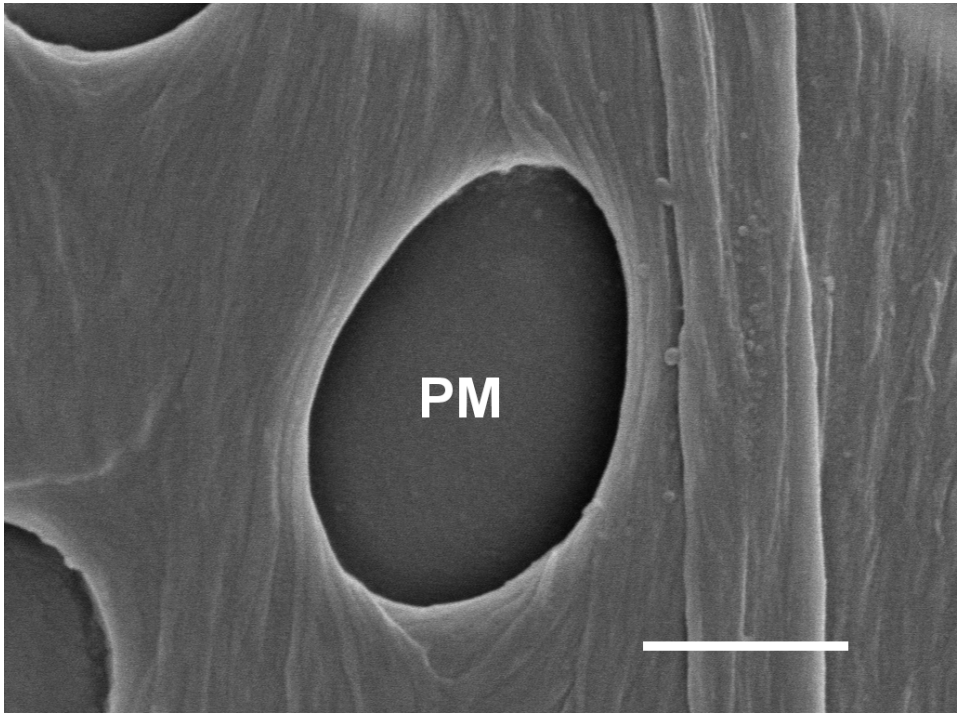
A primary route for spread of the infection,
or the last tissue to be colonized?

Movement within the xylem

- Can the bacterium move systemically within the xylem?



Taiz and Zeiger 2010

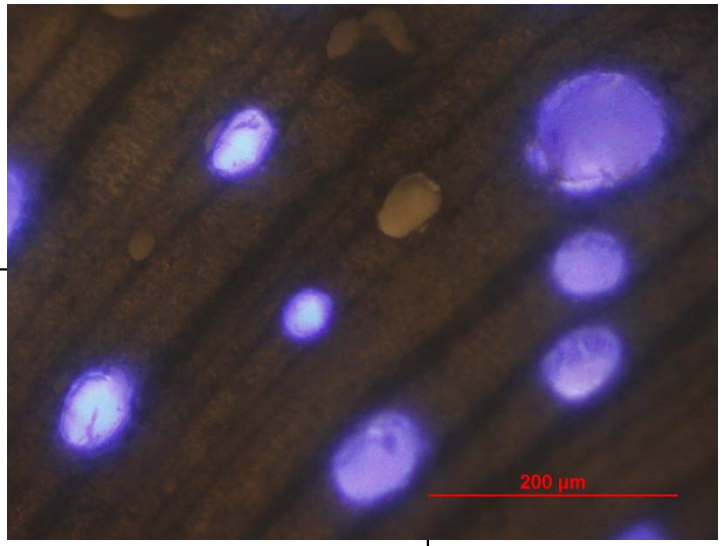


Hort16A kiwifruit vessel pit membrane

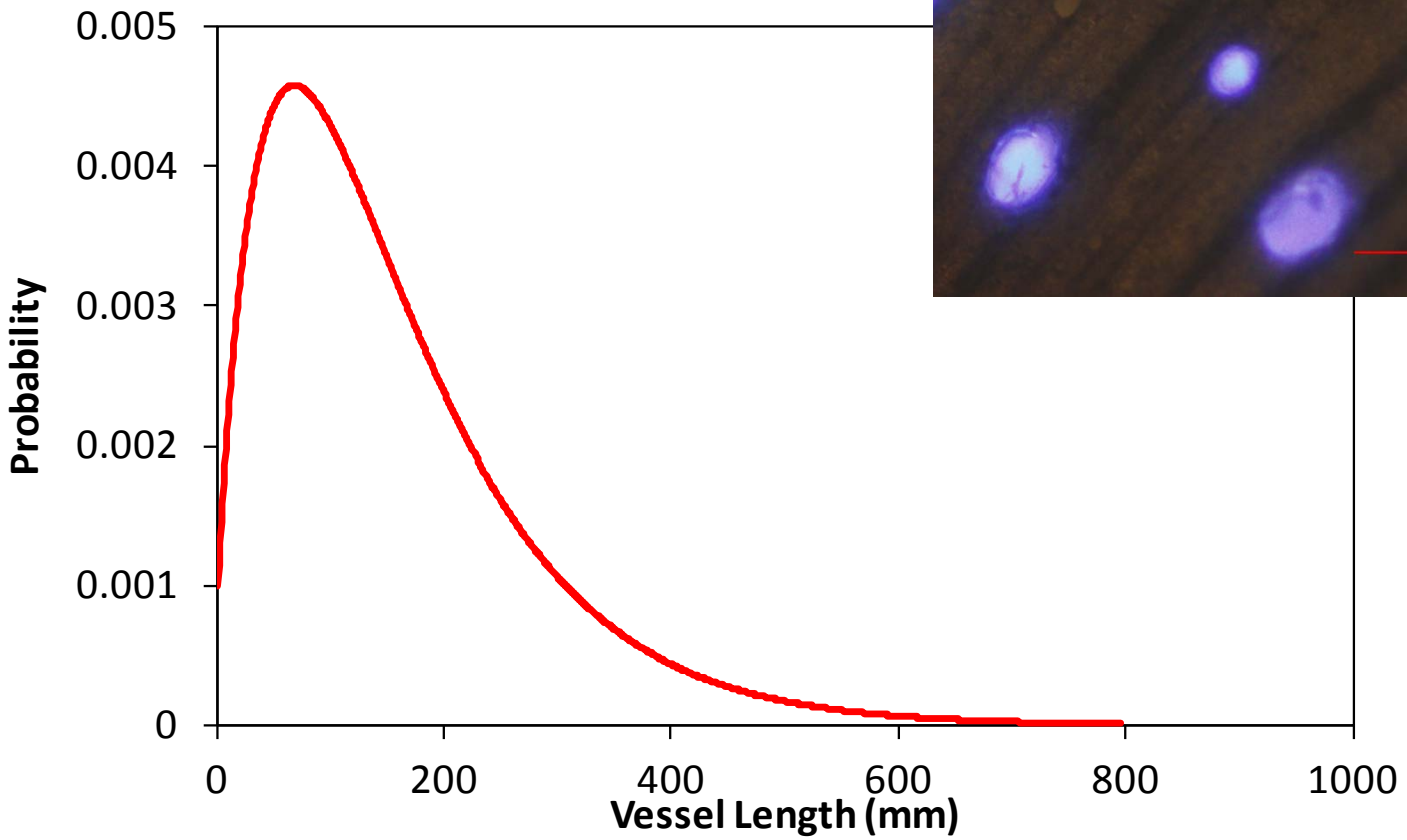
- Movement within the xylem should be related to vessel length and the ability to cross the pit membrane

Measuring vessel length

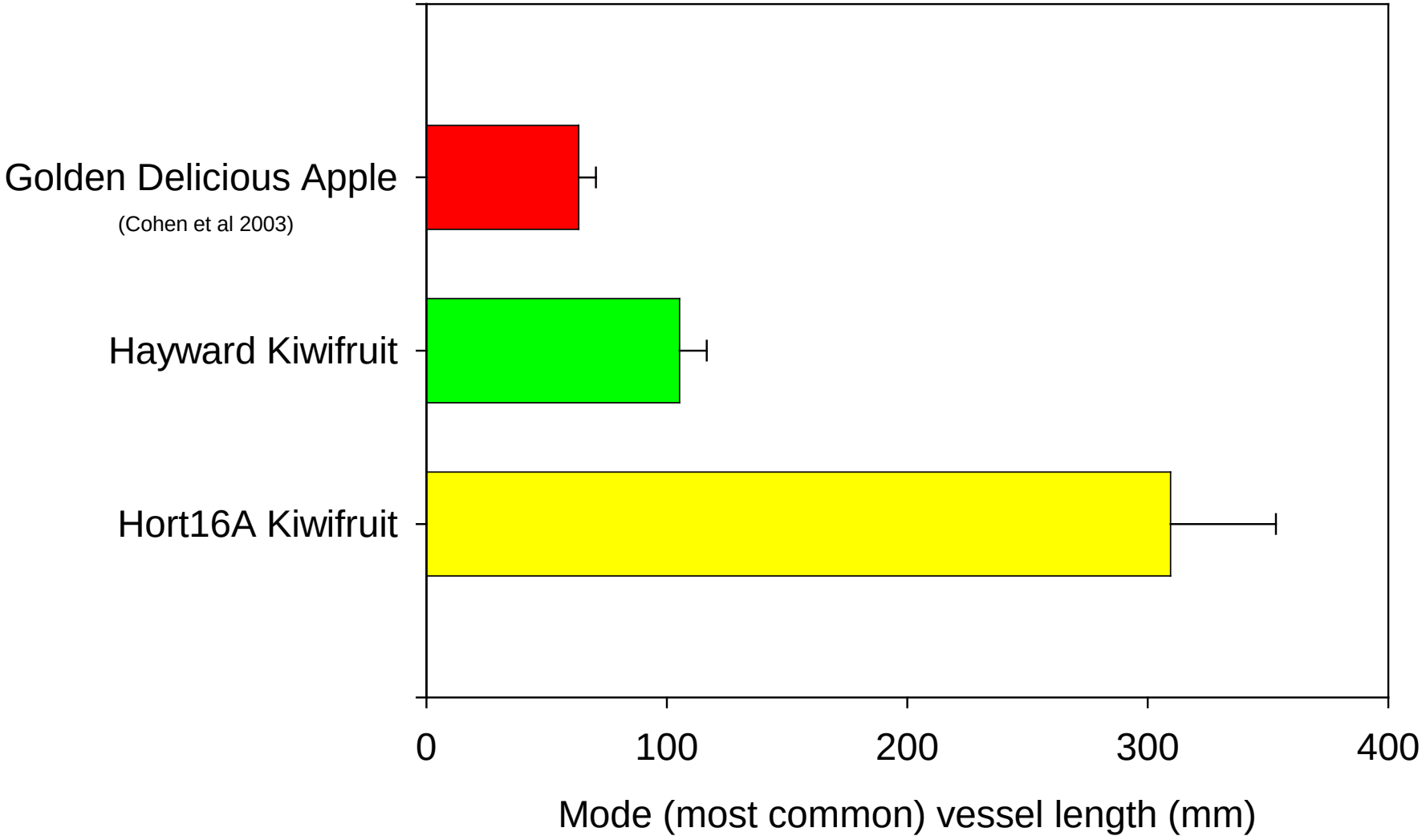
- Air and silicone polymer injection



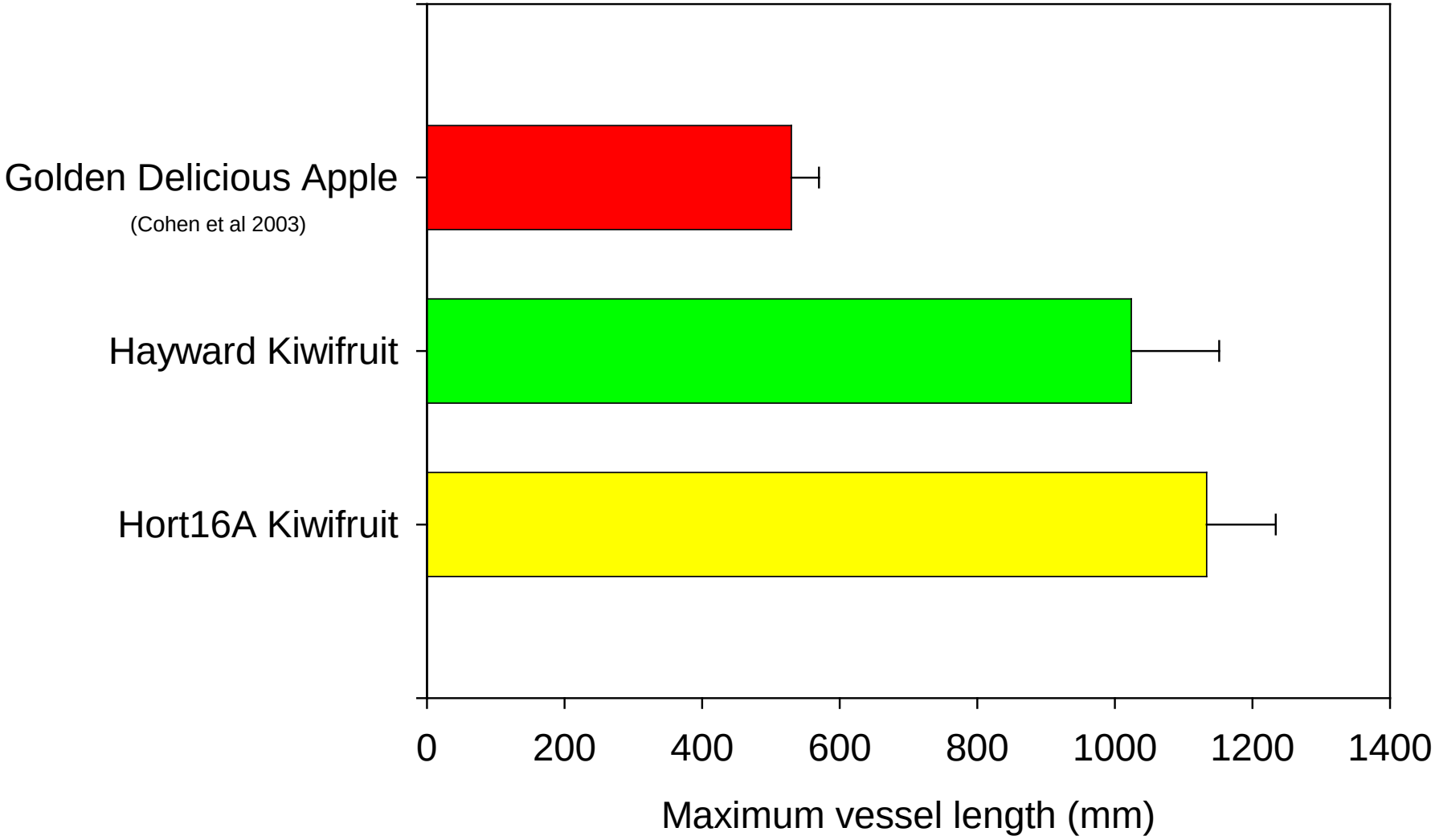
Yanchang Wang



Vessel length in Hort16A and Hayward

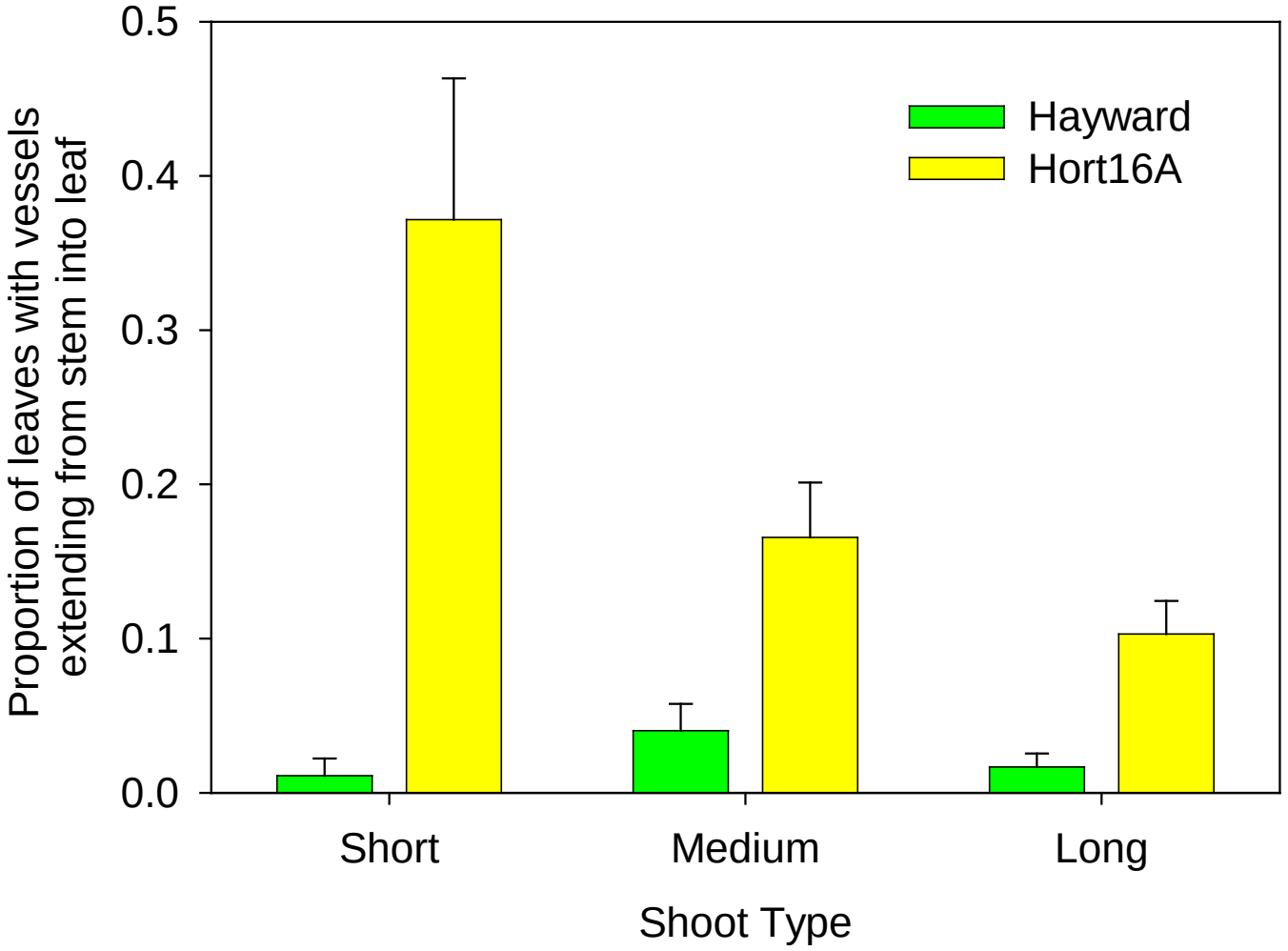


Maximum Vessel Length



- Vessels can be up to 2 m long in one year old canes

Vessel connections between stem and leaves



Proportion of leaves with a continuous vessel extending from base of stem into the leaf petiole or blade

Movement within the xylem

- Vessels are exceptionally long in kiwifruit
- Vessels are longer in Hort16A
- Open vessels between leaf and stem are more common in Hort16A

Conclusion: Clear potential for long distance movement of the pathogen within the xylem, xylem connectivity may contribute to susceptibility

Next steps:

- Further describe vessel connections between leaves and stem
- Track actual bacterial movement within the xylem
- Investigate bacterial capacity to degrade the pit membranes

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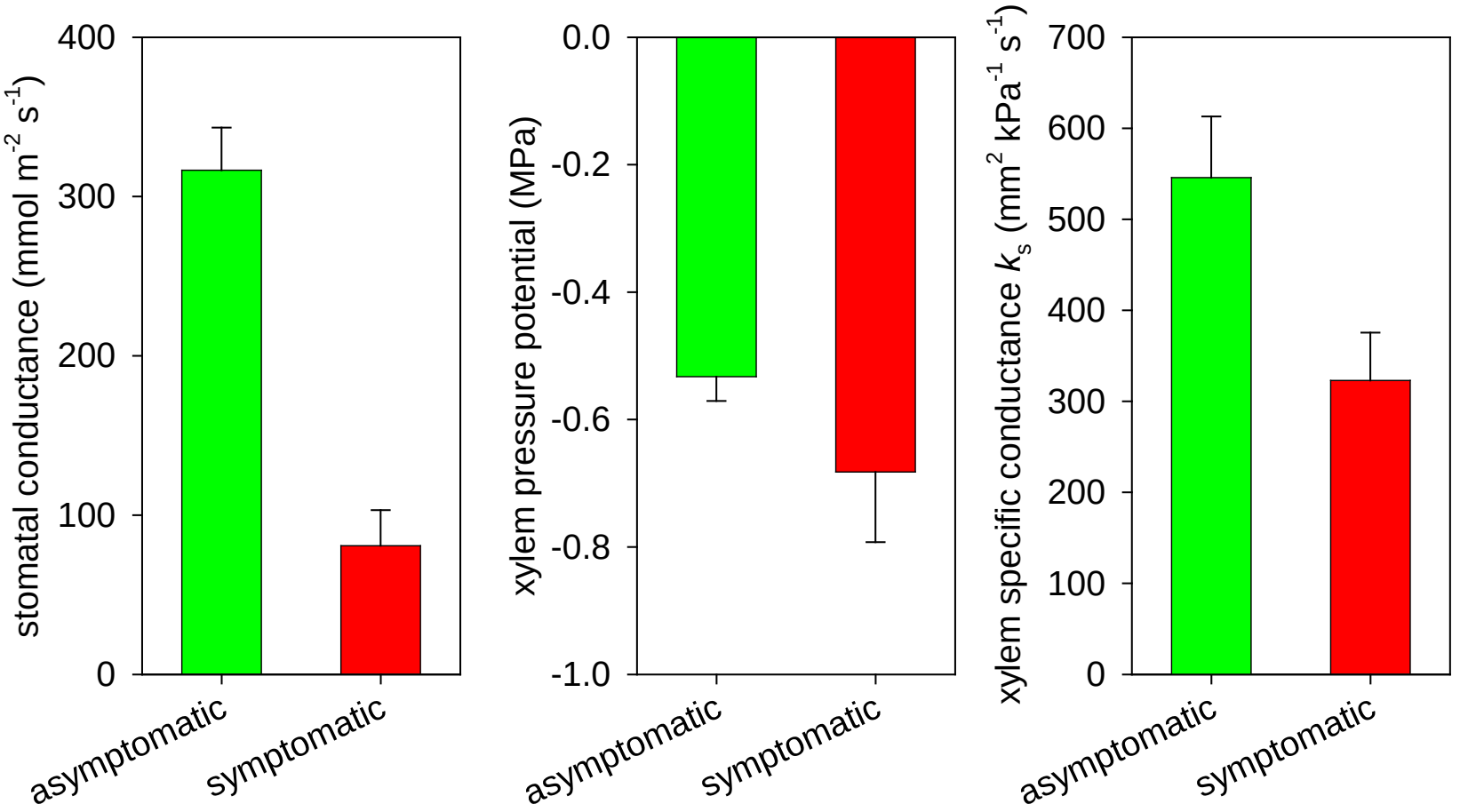
Impact on xylem hydraulic functioning

Is wilting and shoot collapse caused by:

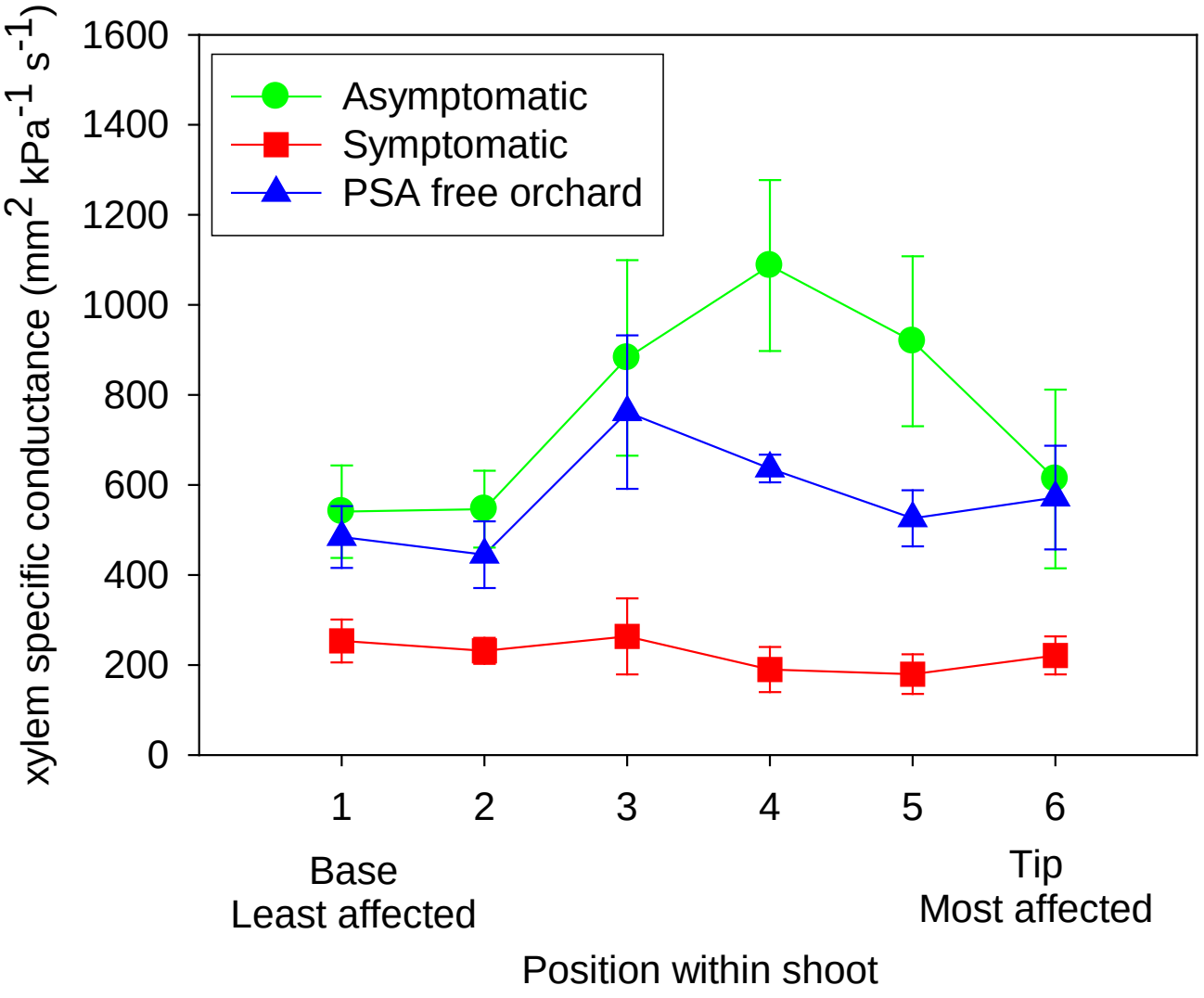
- Bacterial toxins?
- Bacteria blocking the xylem?
- A plant wound-response that blocks the xylem?

Water supply to symptomatic shoots decreases

Compared collapsing and apparently unaffected shoots from the same Hort16A vines



Xylem hydraulic conductance is reduced in collapsing shoots



Mid-portions of shoots divided into six 150 mm long

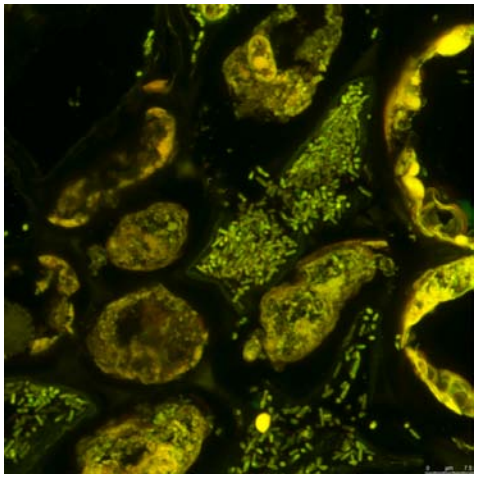
Impact on xylem hydraulic functioning

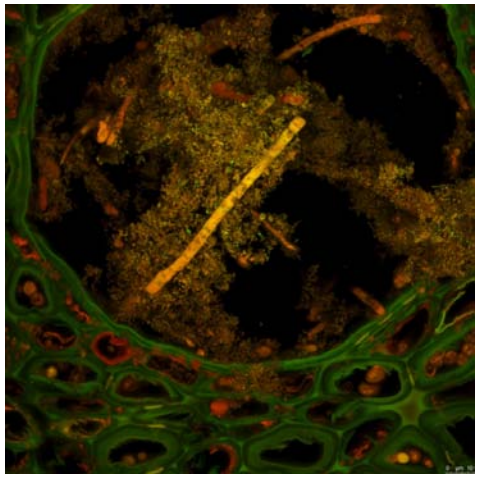
- Infection causes a reduction in xylem hydraulic conductance
- Conductance is reduced before obvious tissue browning and collapse

Conclusion: Reduced xylem hydraulic conductance occurs early, invasion and spread in the xylem may be important.

Next steps:

- Identify cause of blockage in stem samples using microscopy
- Develop hybridization protocol to identify PSAv bacteria *in-situ*





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Overall Conclusions

- Virulence may be connected to conditions in the apoplast, and the ability of PSAv to manipulate those conditions

- Kiwifruit stems have long vessels and vessel connections between leaves and stems that will facilitate faster systemic movement of bacteria that enter the xylem

- A decline in xylem hydraulic conductance is a significant component of the pathology of the disease

Contact

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