A microsatellite analysis identifies global pathways of movement of *Phytophthora cinnamomi* and the likely sources of wildland infestations in California and Mexico

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Phytophthora cinnamomi (Pc)

- One of the first pathogens to be transported and introduced globally in the Anthropocene
- One of the 100 most invasive organisms, relevant both in agriculture and in natural ecosystems around the world
- In North America wildlands, older introduction on the East coast, affecting pines and chestnuts
- More recent wildland introductions in Mexico and California, affecting oaks, manzanita, pacific madrone, California bay laurel

Pc causes 100% mortality on two manzanita spp. in Ione (CA)

Pc killing drought stressed oaks in So-cal (27% infected of 474 tested) in areas next Avocado orchards

Geographic separation of CA outbreaks

1- White and Ione manzanita ; 100% mortality in large area of Sierra Nevada foothills. Ione manzanita is an endangered species

2- Pacific madrone, bay laurels and manzanitas in hotspots of disease around the SF Bay Area

3- Significant mortality of coast live oak associated with impact of drought in San Diego Country and in proximity of agricultural land
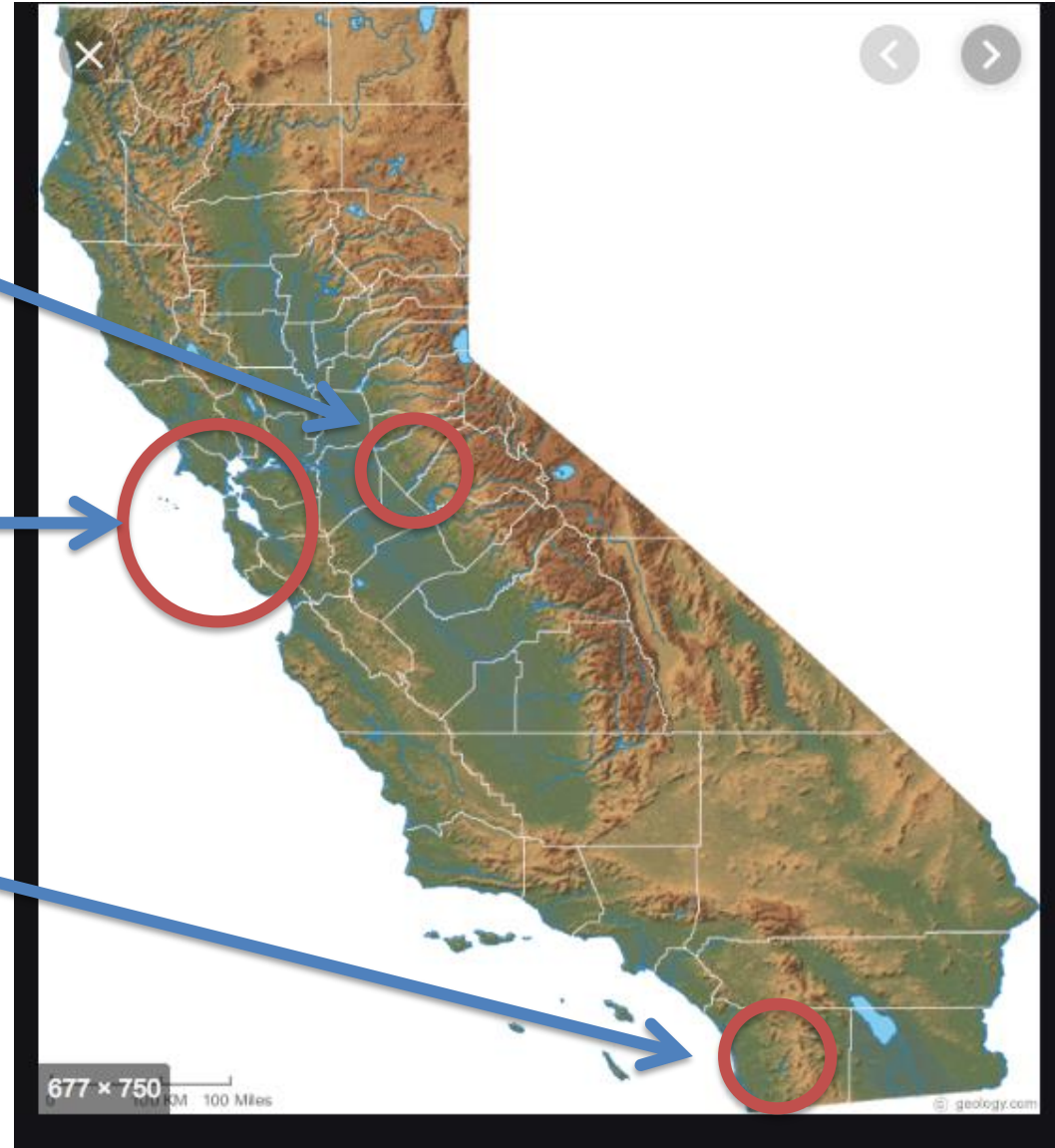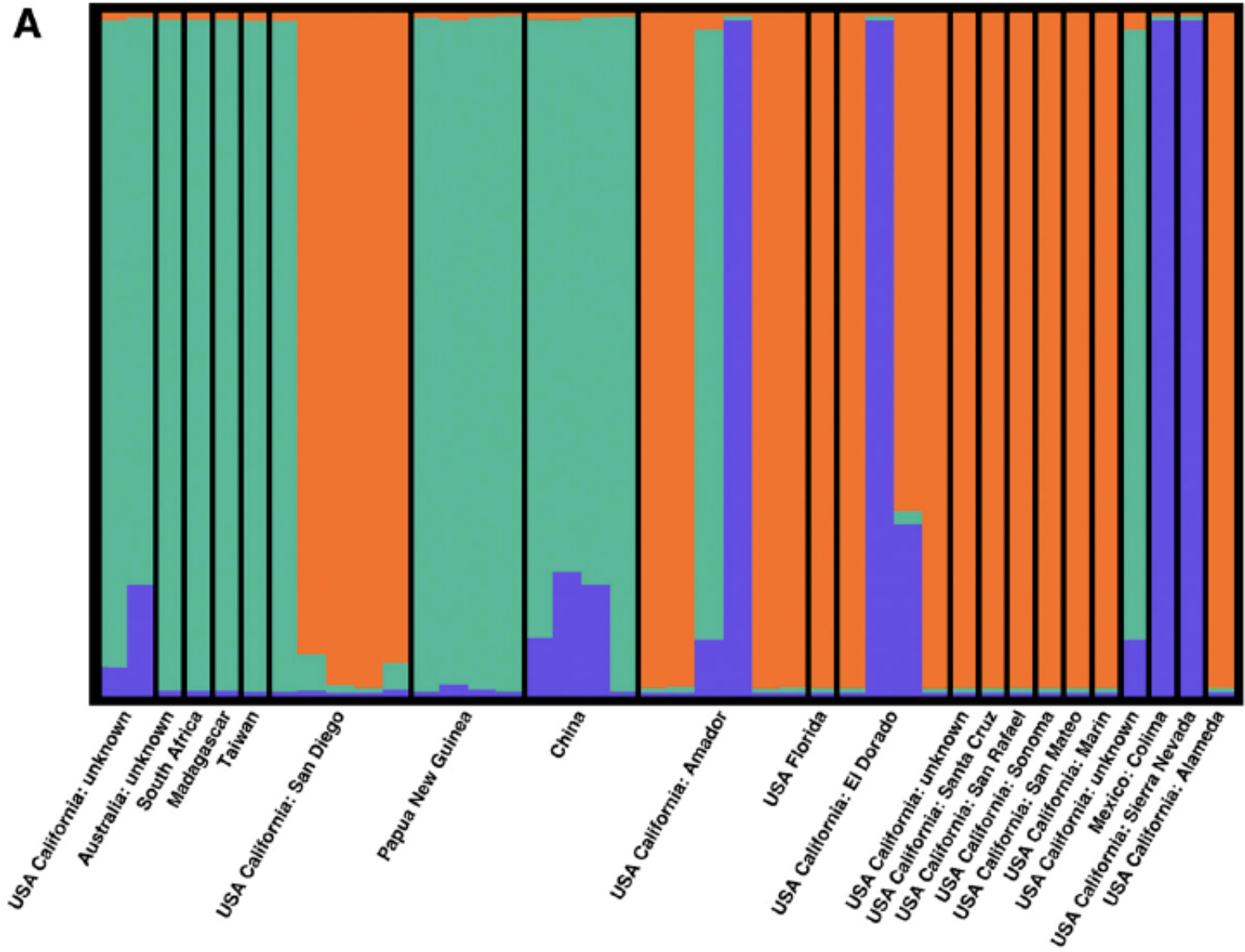
Goals of the study

- 1- Use SSR analysis to identify groups of strains and reconstruct history and pathways of global movement following identical genotypes

- 2- Understand possible sources of introduction into wildlands

- 3- Provide data to identify emergent strains, with enhanced virulence

- 4-Provide data to predict impacts of Pc in California (disease incidence and severity)

History of *Pc* using SSR markers, Bayesian assignment & network analyses

- SSRs (Dobrowoloski et al.) on 159 isolates with a sampling emphasis on CA and MX identified 75 MLGs assigned to three STRUCTURE groups:
  - **WWW1** A1 dominant. All PNG isolates are www1, present also on commodities in Taiwan, China, Australia, South Africa, Florida and California. High genotypic diversity but no sex based on Ia

  - **WWW2**   all A2. Present in MX and CA only. Only one MLG in CA, more diverse and reproducing sexually in MX based on Ia E5 index indicates clonal reproduction significant with some MLGs overrepresented

  - **WWW3** all A2. Present in North America, Sex present, E5 index indicates clonal reproduction significant with some MLGs overrepresented

  - Some MLG are admixed especially www2 x www1 and Ia on all samples indicates recombination

Green= www1 ; Orange = www3; Purple= www2
Each group includes only contiguous MLGs in the MSN

Three MLGs are Emergent or invasive

Switch To A2

Structure group WW1

MG14

MG03

MG01

MG04

MG05

MG16

MG02

Structure group WW3

MG15

MG18

MG17

Structure group WW1

MG10

MG11

MG06

MG07

MG12

MG19

MG09

MG08

Structure group WW2

MG13

MG20

MG23

MG22

MG21

Populations

- ■ USCA_UNKNOWN1
- ■ AUST_UNKNOWN2
- ■ SOAF_SAFRICA
- ■ MADR_MADAGASCAR
- ■ TAIW_TAIWAN
- ■ USCA_SANDIEGO
- ■ PNG_PAP
- ■ CHI_CHINA
- ■ USCA_AMADOR
- ■ USFL_FL
- ■ USCA_ELDORADO
- ■ USCA_UNKNOWN4
- ■ USCA_SANTACRUZ
- ■ USCA_SANRAFAEL
- ■ USCA_SONOMA
- ■ USCA_SANMATEO
- ■ USCA_MARIN
- ■ USCA_UNKNOWN5
- ■ MEX_COLIMA
- ■ USCA_SIERRANEV
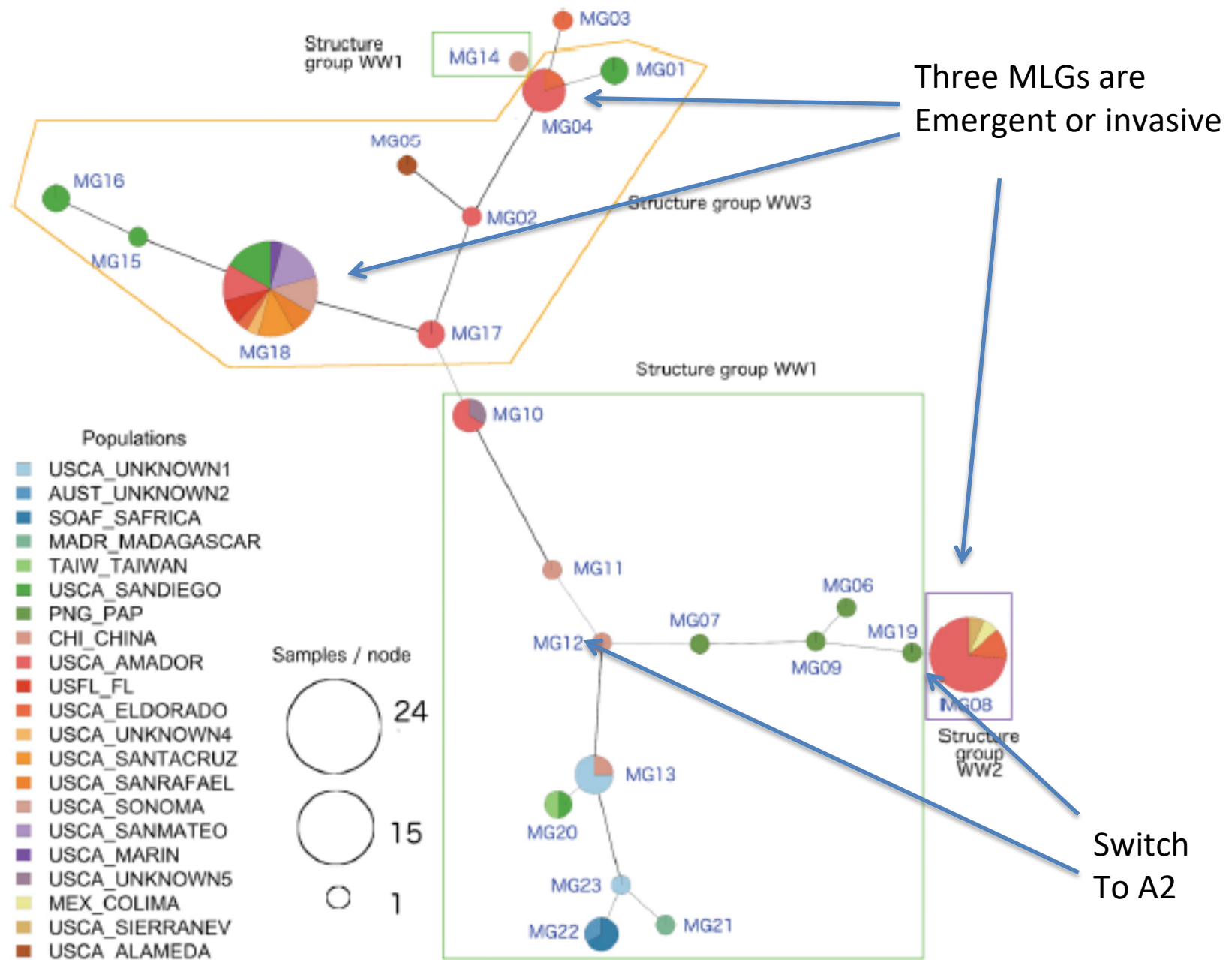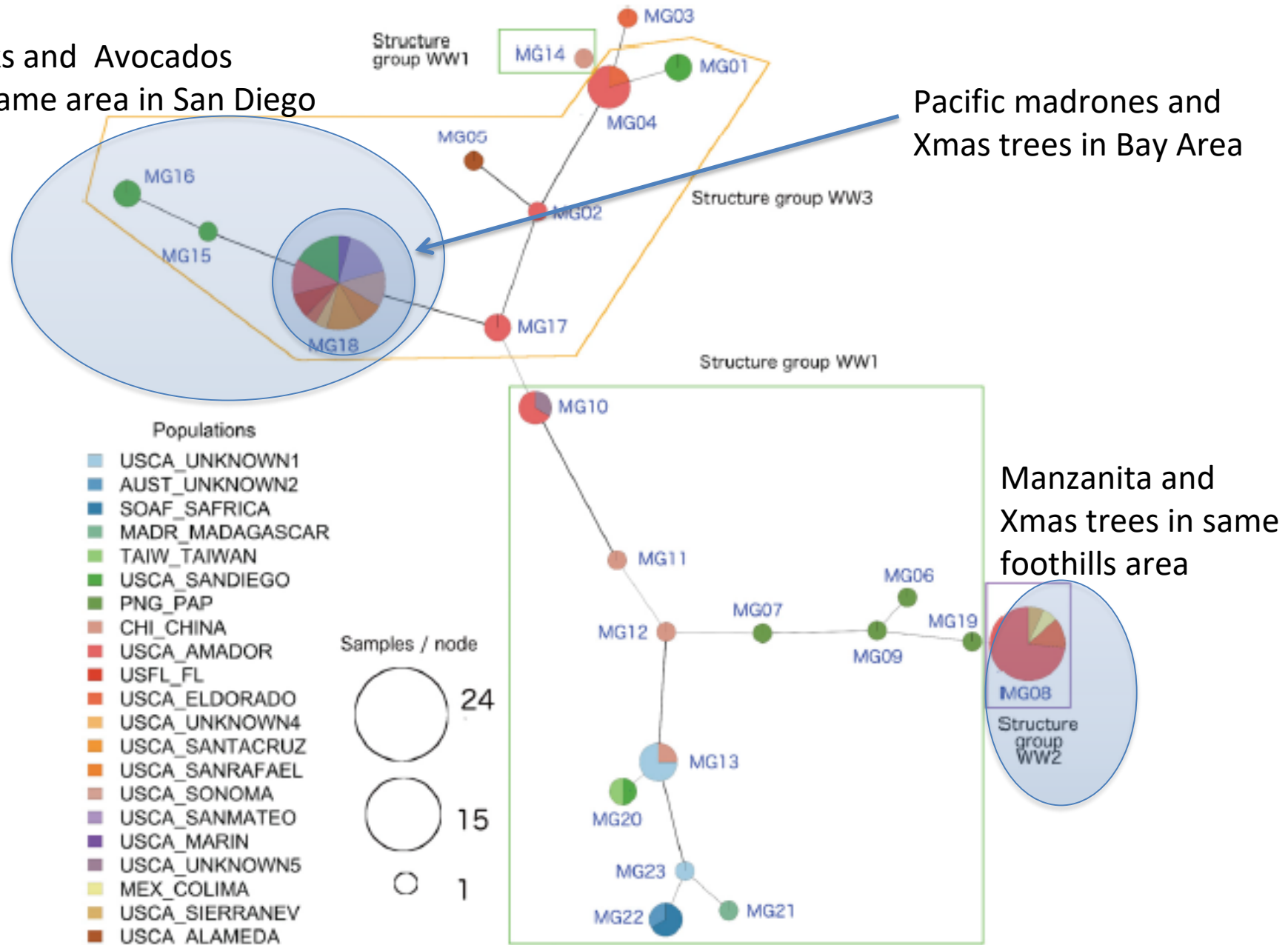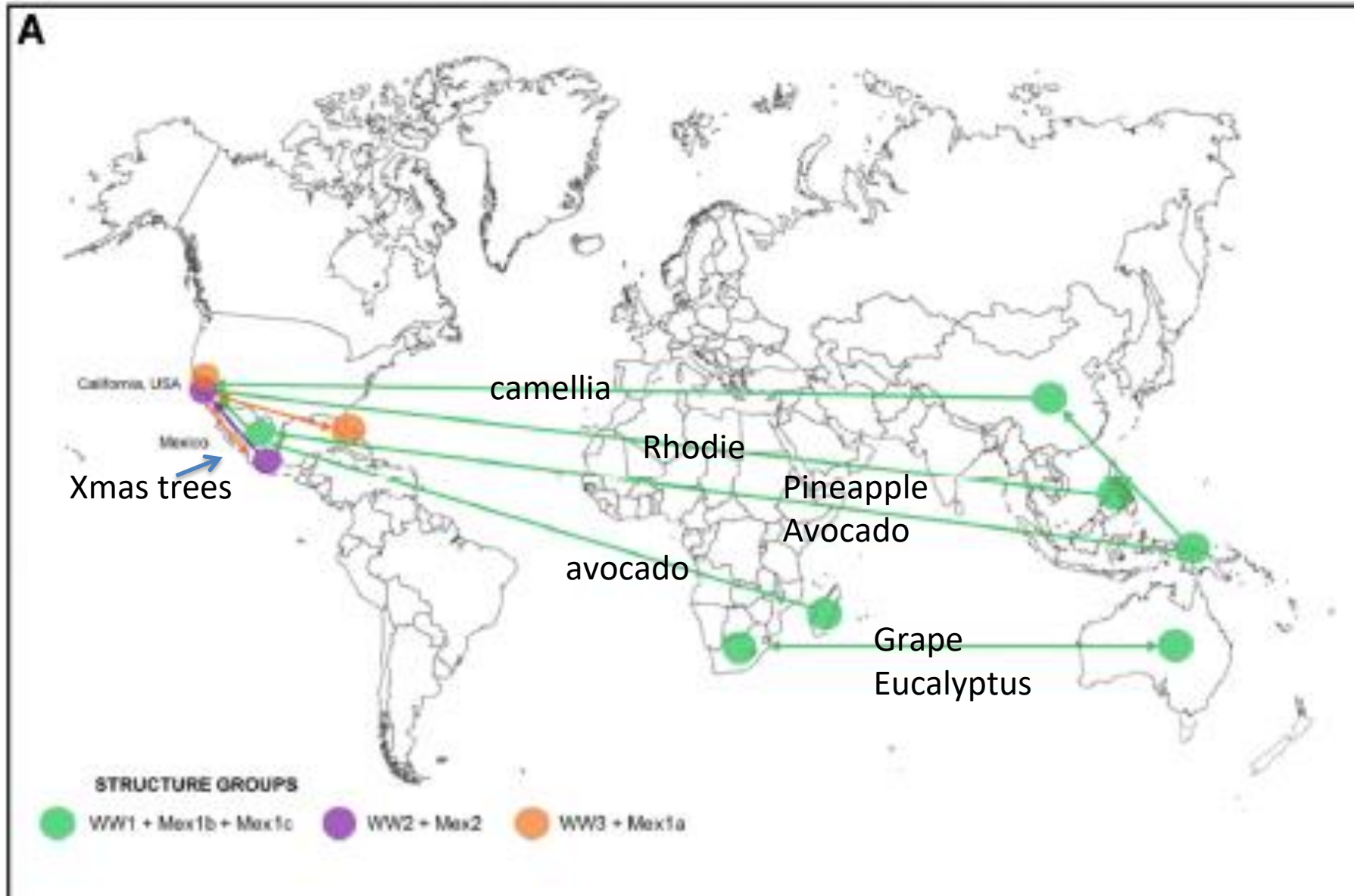- ■ USCA_ALAMEDA
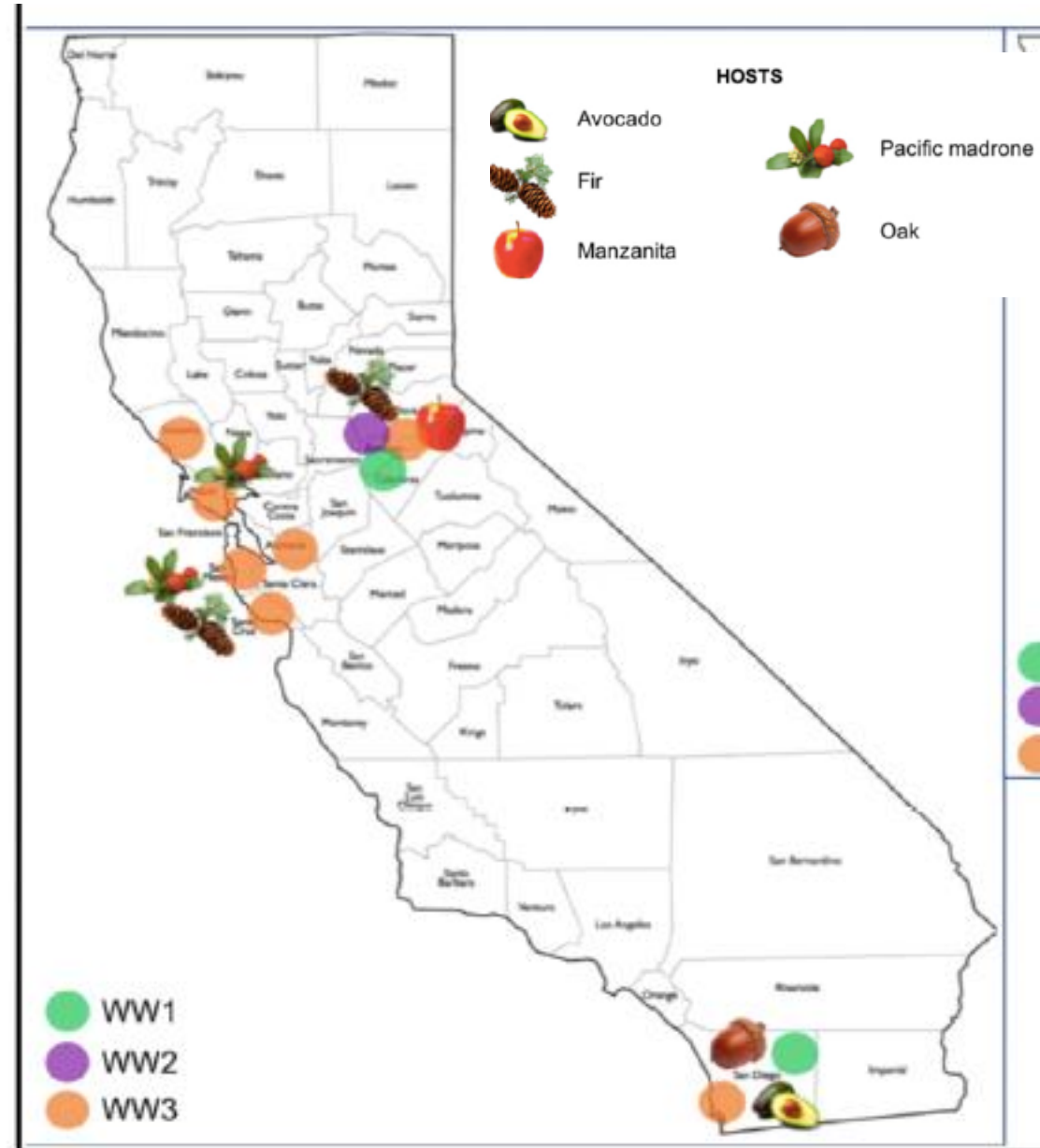
Samples / node

24

15

1

Fig. 3. Minimum spanning network based on the matrix of pairwise Bruvo genetic distances visualizing relatedness among multilocus genotypes (MGs) in the worldwide/California analysis.

Oaks and Avocados In same area in San Diego

Pacific madrones and Xmas trees in Bay Area

Manzanita and Xmas trees in same foothills area

Structure group WW1

Structure group WW3

Structure group WW1

Structure group WW2

MG03
MG14
MG01
MG04
MG05
MG16
MG15
MG02
MG17
MG18
MG10
MG11
MG06
MG07
MG12
MG19
MG09
MG08
MG13
MG20
MG23
MG21
MG22

Populations
- USCA_UNKNOWN1
- AUST_UNKNOWN2
- SOAF_SAFRICA
- MADR_MADAGASCAR
- TAIW_TAIWAN
- USCA_SANDIEGO
- PNG_PAP
- CHI_CHINA
- USCA_AMADOR
- USFL_FL
- USCA_ELDORADO
- USCA_UNKNOWN4
- USCA_SANTACRUZ
- USCA_SANRAFAEL
- USCA_SONOMA
- USCA_SANMATEO
- USCA_MARIN
- USCA_UNKNOWN5
- MEX_COLIMA
- USCA_SIERRANEV
- USCA_ALAMEDA

Samples / node
24
15
1

Fig. 3. Minimum spanning network based on the matrix of pairwise Bruvo genetic distances visualizing relatedness among multilocus genotypes (MGs) in the worldwide/California analysis.

Same MLG in different parts of the world. WW1 ancestral

A

California, USA

Mexico

Xmas trees

camellia

Rhodie

Pineapple
Avocado

avocado

Grape
Eucalyptus

STRUCTURE GROUPS

WW1 + Mex1b + Mex1c   WW2 + Mex2   WW3 + Mex1a

1-Number of lineages (1 in Bay Area; 2 in San Diego: 3 in Ione)

2- Same MLG in agriculture and wild host in same region

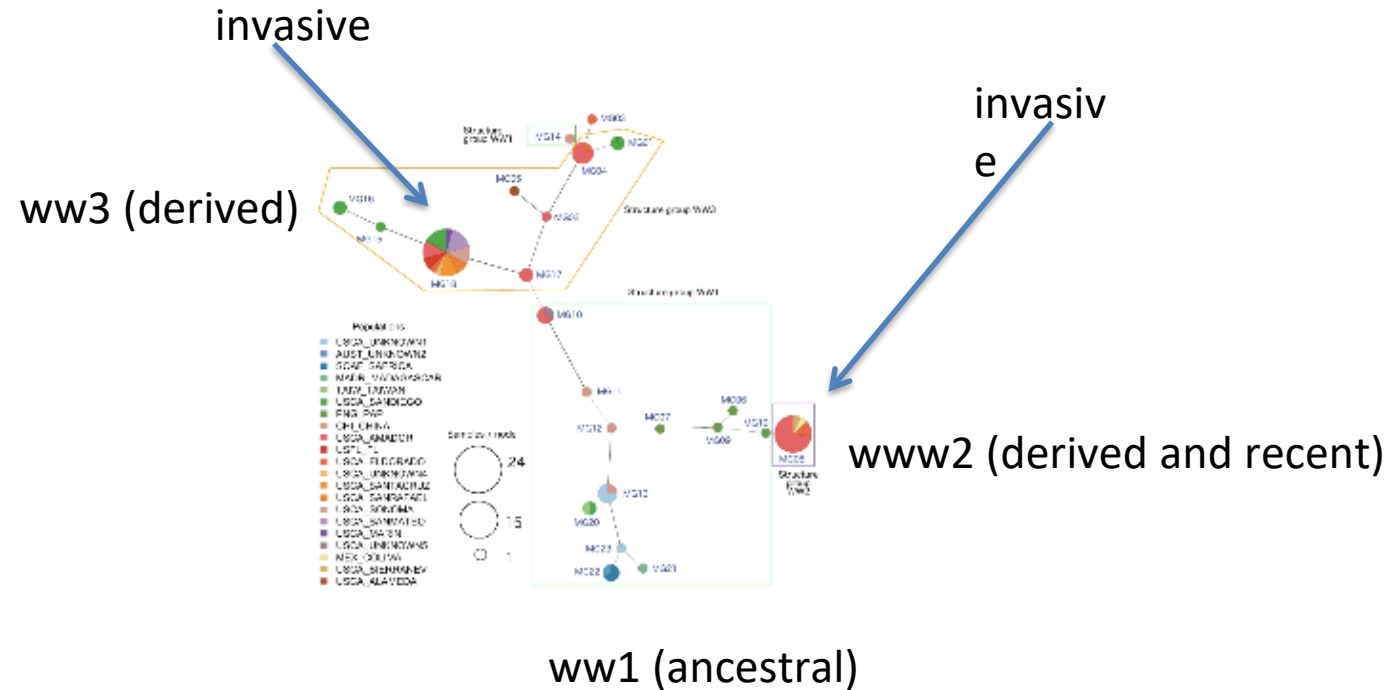# Some strains (represented by circles) are clearly invasive

Fig. 3 Minimum Spanning Network based on the matrix of pairwise Bruvo genetic distances, visualizing relatedness among multi locus genotypes (MGs) in the worldwide/California analysis.

215x279mm (300 x 300 DPI)

- Some recently derived strains are way more abundant than older strains, they are present in different world regions and on different hosts (the bigger the circle the more abundant the strain, dataset only considers one isolate per site)

Severity of foliar symptoms (0-4 scale)
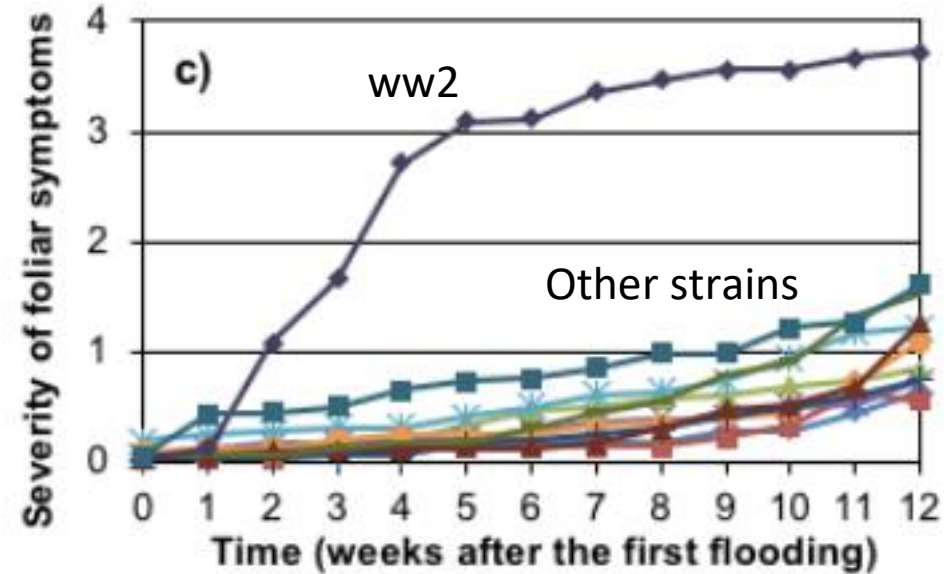

Weekly

At the end of the experiment

Length of the visible stem lesion (external lesion)

Length of the internal stem lesion
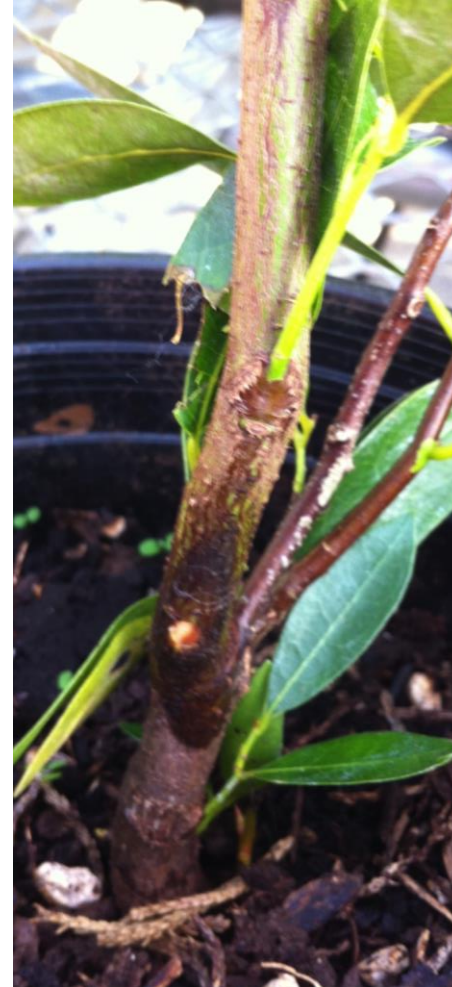
Is WW2 strain more aggressive?
Results from inoculation on bays

Are native hosts equally susceptible? NO!

		Average
Pacific Madrone	rAUDPC[a]	31.8±5.5 A
	PSA	81.8±0.5 A
	% girdling[c]	17.5 [b]
	% mortality[d]	36.6 [c]
Douglas-fir	rAUDPC[a]	14.1±2.8 B
	PSA	19.8±3.9 B
	% girdling[c]	30.0 [b]
	% mortality[d]	1.0 [c]
California bay laurel	rAUDPC[a]	2.8±1.4 C
	PSA	13.8±0.1 C
	% girdling[c]	8.0 [b]
	% mortality[d]	4.0 [c]

15   [a]Severity of foliar symptoms progress curve (rAUDPC)

16   PSA= Percentage Stem affected

Introduction of Pc in an ecosystem is going to have different impacts depending on host

Is there significant host x pathogen genotype interaction (Douglas-fir)

- Most aggressive isolate on roots and on stems not the same
- Nursery isolate most aggressive on stems
- PNG isolate most aggressive on roots
- Introducing both= strong impact on host

Stem inoculations

Root inoculations
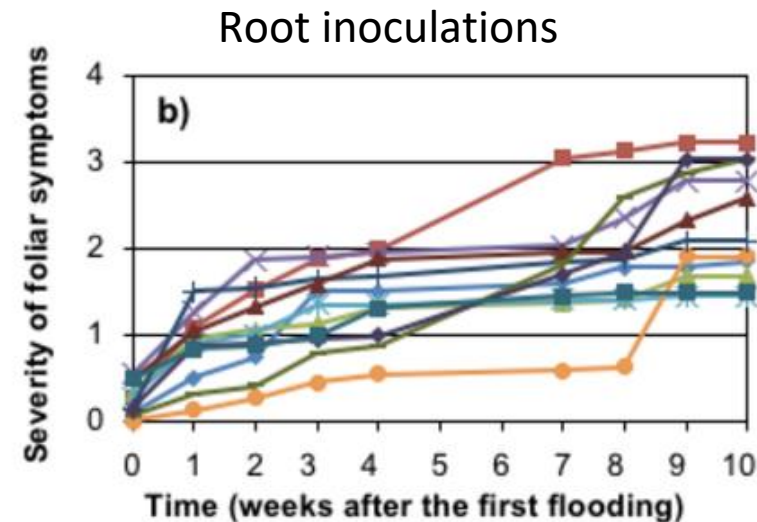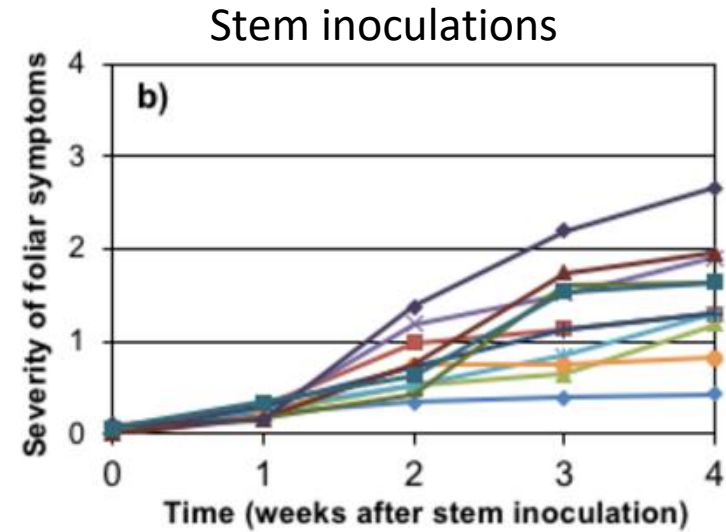
Conclusions

- Spread history of PC partially reconstructed and identified some commodities responsible for global spread and for release of Pc in nature
- Different strains in different wildlands
- Some strains are emergent and more aggressive
- Some hosts are more susceptible, host x strain interaction found
- **Should we prevent both the spread of the pathogen and the spread of strains with known higher virulence?**

Thank you