The Intersection of Climate Change, Anthropogenic Pressure, and Emergent Diseases is a Major Threat to California

Matteo Garbelotto

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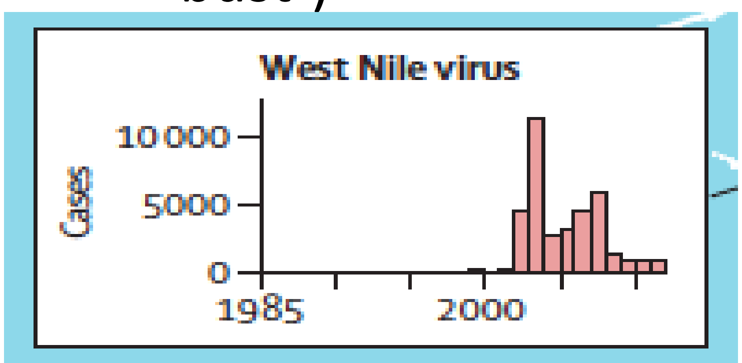
EMERGENT DISEASES

- Caused by <u>exotic pathogens</u> introduced from a different region of the world.

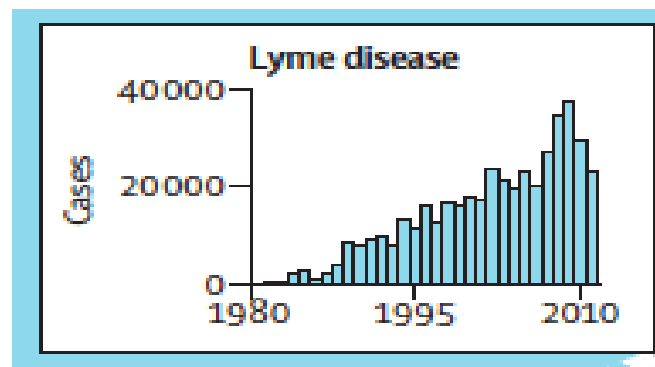
- Caused by climatic or ecological changes increasing pathogenicity of <u>native microbes</u>
  - Global warming, strongest effects are at the margin of tree ranges
  - Anthropogenic alteration of the ecosystem
  - Exotic ecosystem and native pathogens: planting of exotics or planting off site

Emergent Diseases: temporal patterns are generally different between:

- EXOTIC AGENTS
  - Rapid outbreaks
  - May cycle down after outbreak ("boom and bust')



- NATIVE AGENTS
  - Progressive, gradual even if dramatic increase
  - Less likely to cycle down



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Human Domination of Earth's Ecosystems

Peter M. Vitousek, Harold A. Mooney, Jane Lubchenco, Jerry M. Melillo

Human alteration of Earth is substantial and growing. Between one-third and one-half of the land surface has been transformed by human action; the carbon dioxide concentration in the atmosphere has increased by nearly 30 percent since the beginning of the Industrial Revolution; more atmospheric nitrogen is fixed by humanity than by all natural terrestrial sources combined; more than half of all accessible surface fresh water is put to use by humanity; and about one-quarter of the bird species on Earth have been driven to extinction. By these and other standards, it is clear that we live on a human-dominated planet.

interact with the atmosphere, with aquatic systems, and with surrounding land. Moreover, land transformation interacts strongly with most other components of global environmental change.

The measurement of land transformation on a global scale is challenging; changes can be measured more or less straightforwardly at a given site, but it is difficult to

- **Climate Change, Urbanization/Direct Anthropogenic Effects and Biological Invasions** are the top three causes of loss of biodiversity on earth in the Anthropocene

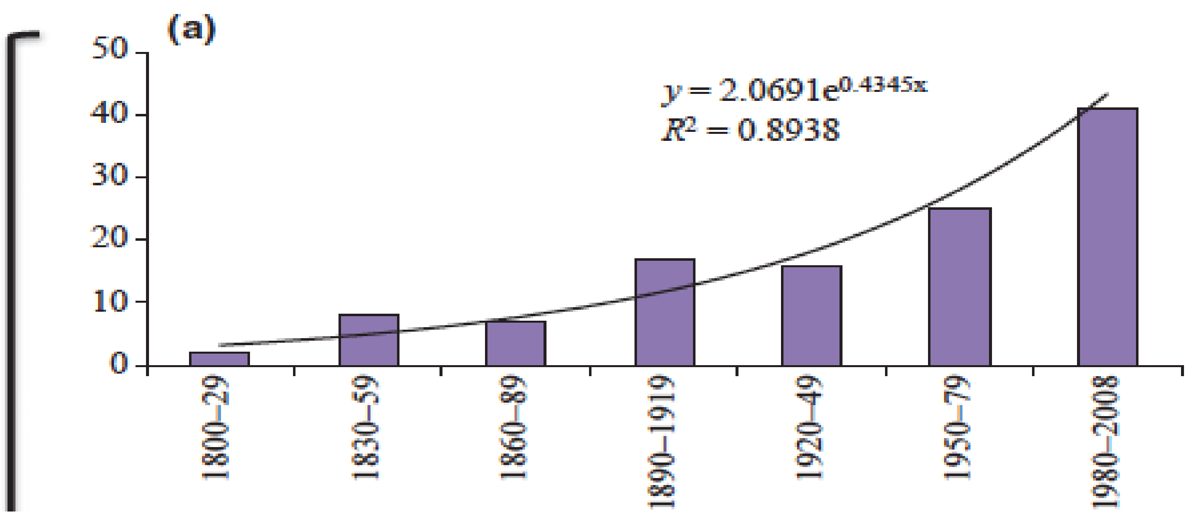
New Phytologist

Biogeographical patterns and determinants of invasion by forest pathogens in Europe

2013

A. Santini[1], L. Ghelardini[1], C.De Pace[2], M. L. Desprez-Loustau[3], P. Capretti[4], A. Chandelier[5], T. Cech[6], D. Chira[7], S. Diamandis[8], T. Gaitniekis[9], J. Hantula[10], O. Holdenrieder[11], L. Jankovsky[12], T. Jung[13], D. Jurc[14], T. Kirisits[15], A. Kunca[16], V. Lygis[17], M. Malecka[18], B. Marcais[19], S. Schmitz[5], J. Schumacher[20], H. Solheim[21], A. Solla[22], I. Szabò[23], P. Tsopelas[24], A. Vannini[25], A. M. Vettraino[25], J. Webber[26], S. Woodward[27] and J. Stenlid[28]

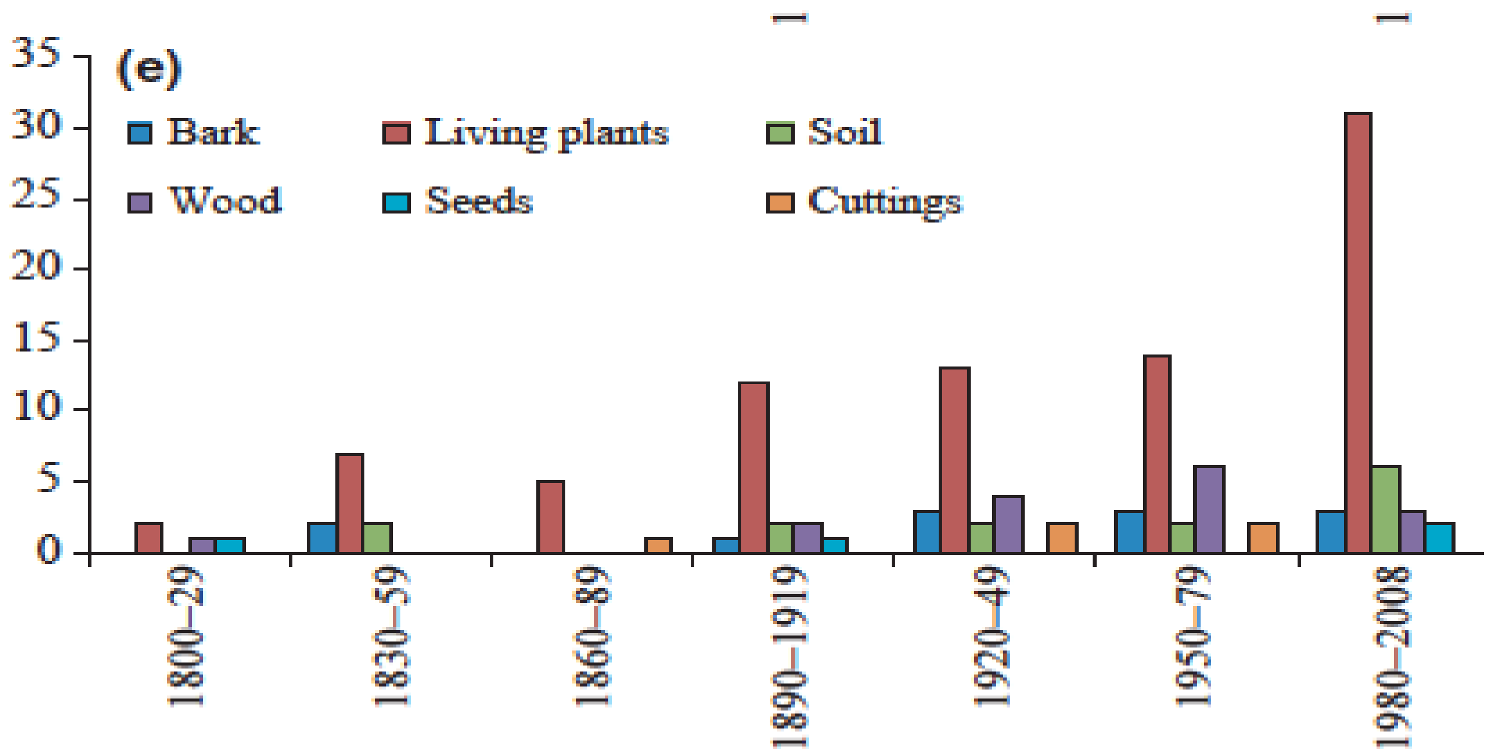
Invasive Forest Pathogens



Globalization: more and faster trade

Figure 2: The global aviation network

SUBSTRATE/PATHWAY of introduction



Bases of Invasion Biology of Pathogens

- Establishment success is related to
  - presence of host (if host specific) or of similar host
  - survival as saprobe or thanks to resting structures
  - similarity in climate between native home and new region
  - lack of competitors/predators
  - number of introductions

- If transmission > mortality then organism becomes invasive (Rt>1)

Presence of host

- SYSTEMATIC INTRODUCTIONS
  - Worse scenario: we introduce exotics in natural ecosystems (soilborne Phytophthoras in restoration sites)
  - Intermediate: introduced through ornamental plants (escape from gardens and nurseries: Sudden Oak Death)
  - “Best”: Introduced in agricultural settings: *Phytophthora cinnamomi*

Exotic *Phytophthora* species are being systematically introduced in California wildlands during restoration projects

Laura Lee Sims & Matteo Garbelotto

Exotic soilborne Phytophthoras can devastate California Native Ecosystems

REVIEW ARTICLE

Soil- and waterborne *Phytophthora* species linked to recent outbreaks in Northern California restoration sites

A review identifies several *Phytophthora* species found in California wildlands and discusses approaches for preventing and diagnosing the spread of these plant pathogens.

by Matteo Garbelotto, Susan J. Frankel and Bruno Scanu

Genus of plant pathogens also known as water molds, not fungi but in same kingdom as Kelp. Normally soilborne and waterborne

- We need to provide rigorous evidence these microbes are truly being introduced through restoration efforts.

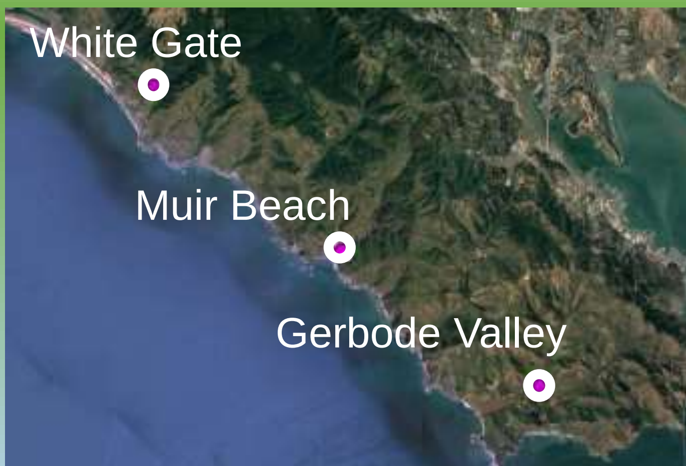
- We need to identify the pathway of introduction, e.g. is it because of the use of infected plant stock coming from infested plant production facilities?

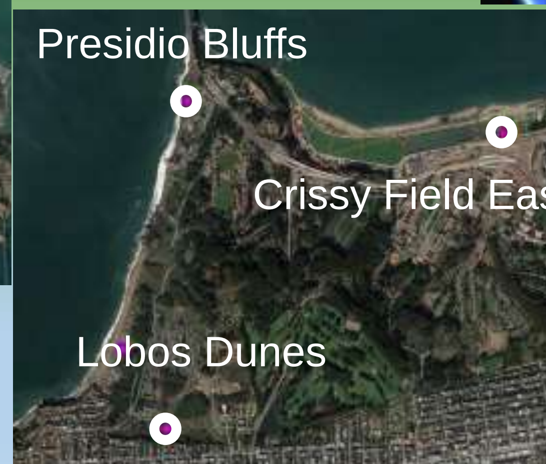
Field sampling approach

- Three types of nearby plots
  - planted **restored**
  - unplanted **undisturbed**
  - unplanted **disturbed**: trails, roads, stream inundation connecting restoration
- From upland and lowland areas where possible
- Compare to nursery results

Locations: 9 sites in 3 counties

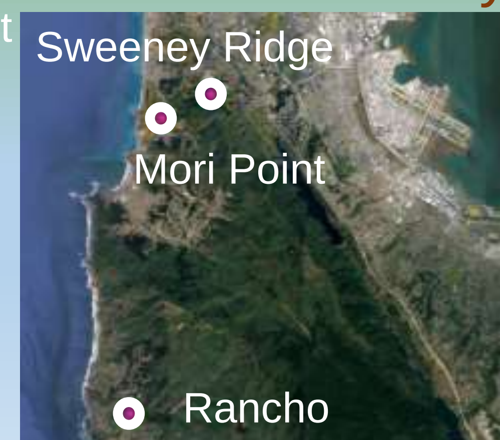
Marin County





San Francisco County

San Mateo County



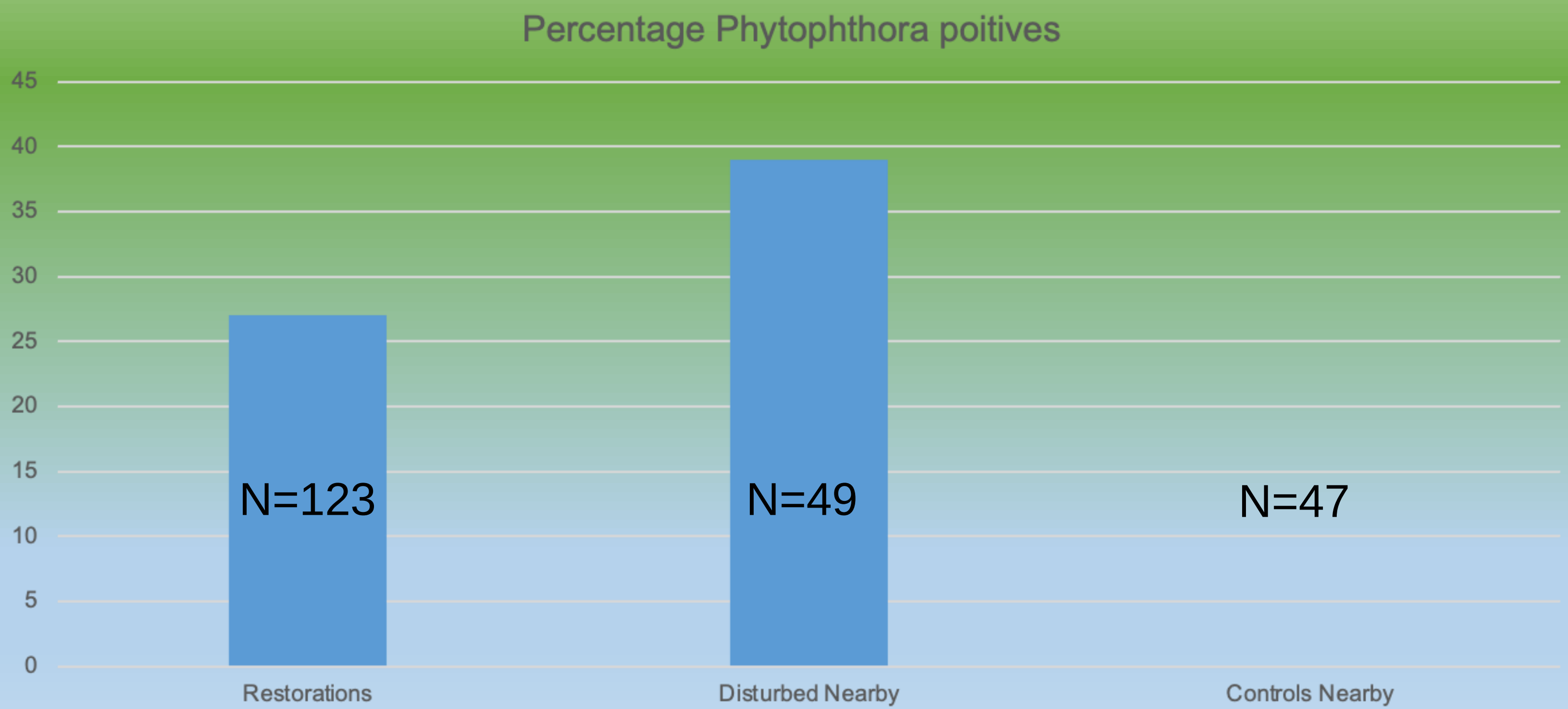
Results field isolations

- Isolation success was highest from three native plant species widely used in restoration projects
  - *Diplacus aurantiacus* (sticky monkey-flower)
  - *Ceanothus thyrsiflorus* (blueblossum)
  - *Frangula californica* (California coffeeberry)

- Isolation success was higher from lowlands (expected from soilborne/waterborne pathogens)

- *Phytophthora crassamura* was always the most abundant (range 1.5 to 10 times, depending on County). Only described from Italy!

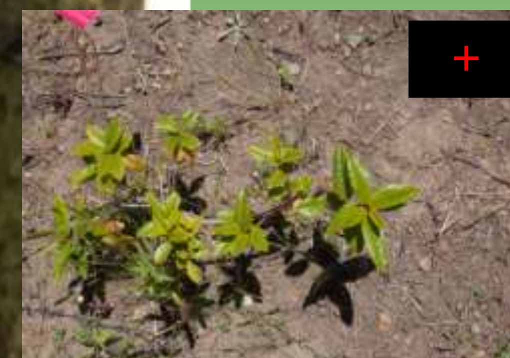
Phytophthoras were present in restorations and in nearby sites disturbed by roads, culverts, etc., but not in nearby undisturbed sites!



Upland *Diplacus aurantiacus*

Upland *Ceanothus thyrsiflorus*

Lowland *Frangula californica*



Thanks to our comparative survey of restored, disturbed and untouched sites, we clearly show that multiple exotic *Phytophthora* species are associated with restoration efforts and the same species are further spread by disturbances (soil and water movement)

Nurseries and Wildlands: *Phytophthora* species found on same host

Sticky monkey flower

Diplacus aurantiacus

n=126 samples =20 nursery + 106 wildland

Nurseries and Wildlands:

- ***P. pseudocryptogea***
- ***P.* taxon kelmania**
- ***P. crassamura***

Wildlands only:

- *P. megasperma*

Nurseries only:

- *P*. taxon kelmania-type 2

California coffeeberry

Frangula californica

n=91 samples =30 nursery + 61 wildland

Nurseries and Wildlands:

- ***P. crassamura***
- ***P. multivora***
- ***P. pseudocryptogea***

Wildland only :

- *P. megasperma*
- *P*. taxon kelmania
- *P. inundata*

Nurseries only:

- *P. cactorum*
- *P. hedraiandra* X *cactorum*

Blueblossum

Ceanothus thyrsiflorus

n=45 samples =30 nursery + 15 wildland

Nurseries and Wildlands:

- ***P. multivora***

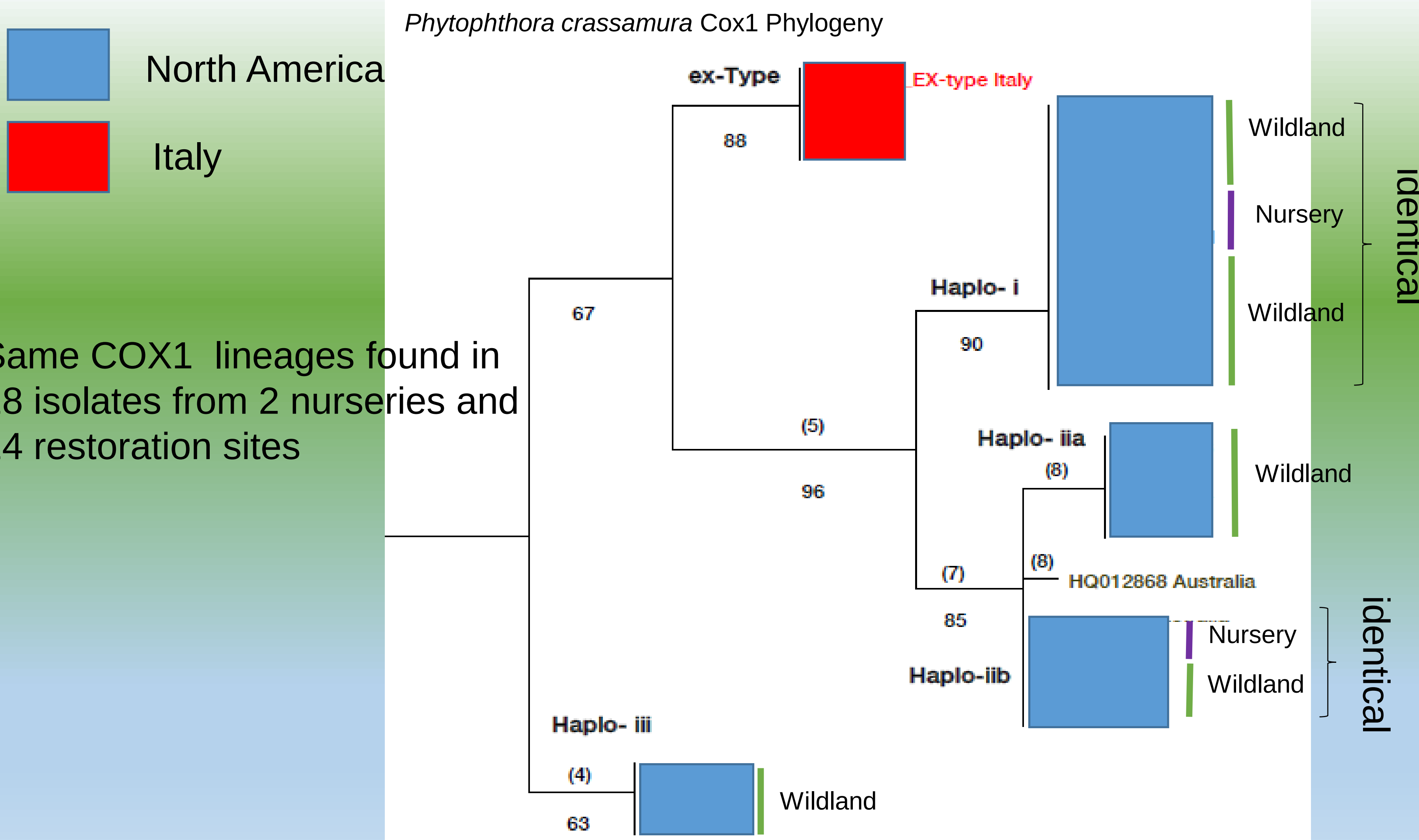
Wildlands only:

- *P. pseudocryptogea*

Nurseries only:

- *P. cactorum*
- *P. hedraiandra*
- *P. niederhauserii*

So are the *Phytophthora* strains in wildlands the same as the ones in nurseries: the case of *P. crassamura*



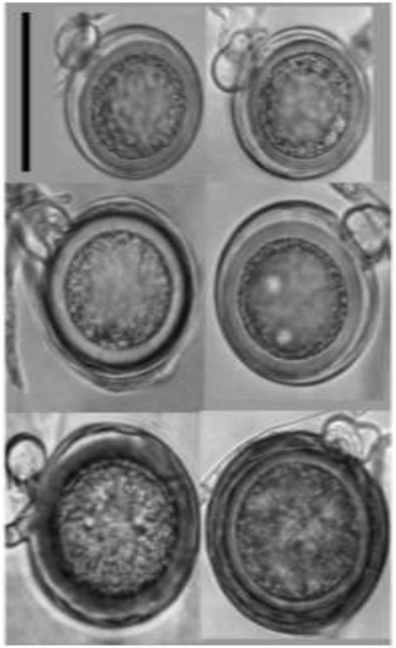
Same COX1 lineages found in 28 isolates from 2 nurseries and 14 restoration sites

Sims, L.L et al. 2019. *Fungal Biology*

What about morphology, resistance to mefenoxam, and virulence?

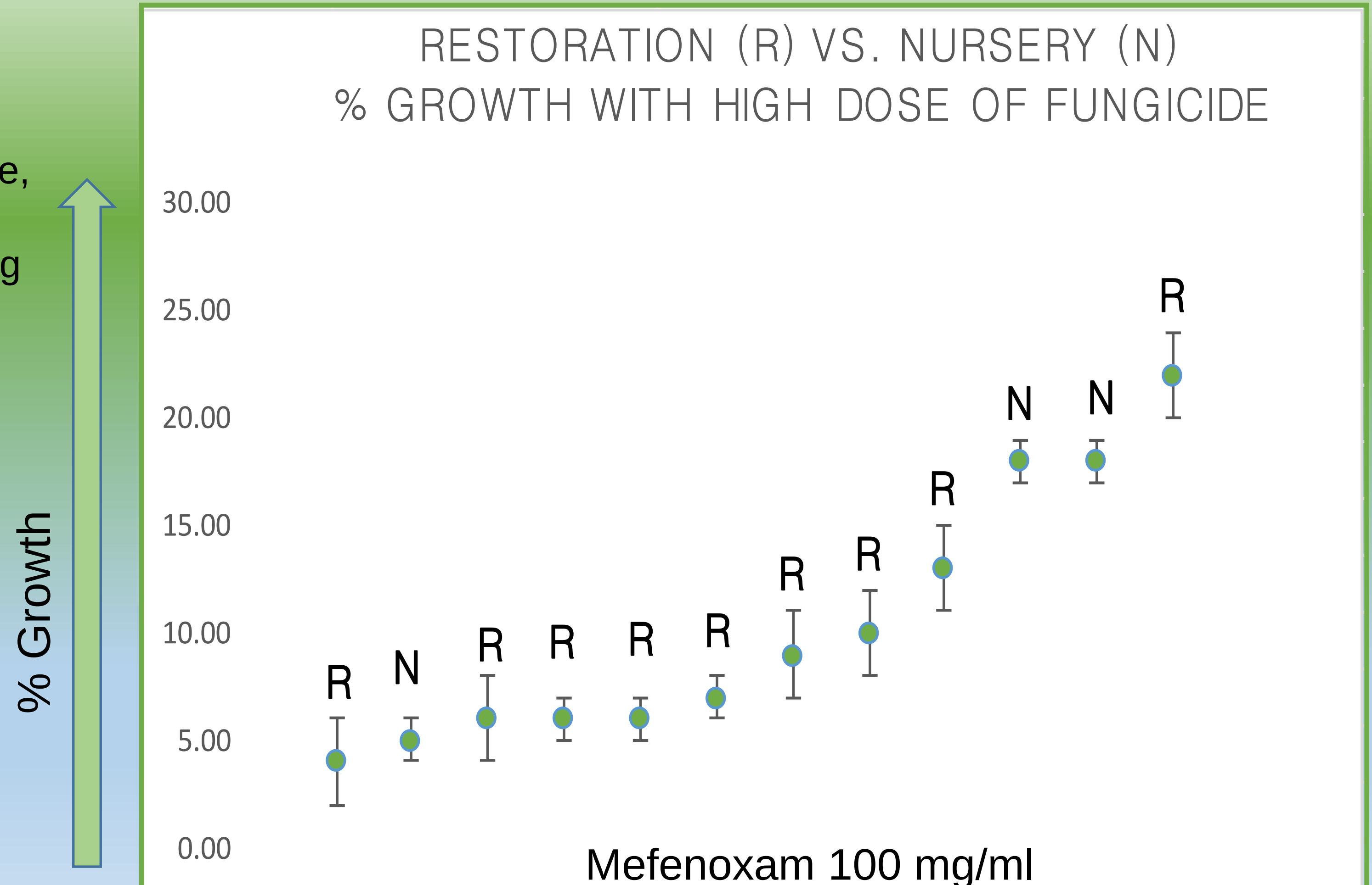
- Phenotypic and genetic data were used to characterize 28 isolates resembling *Phytophthora megasperma* from 14 host species in 2 plant production facilities and 10 restoration sites across the Bay Area
    - Size of the oogonia differentiates lineages and strains in nurseries and restoration had same oogonial size

- Sensitivity to mefenoxam was examined to check for a history of exposure indicated by variable sensitivity and survival for 12 isolates and 3 replicates of each
    - measurements of colonies growing on medium amended with mefenoxam (0, 0.1, 1, 5, 10, and 100 mg/ml)
    - Measurements were taken on d 7



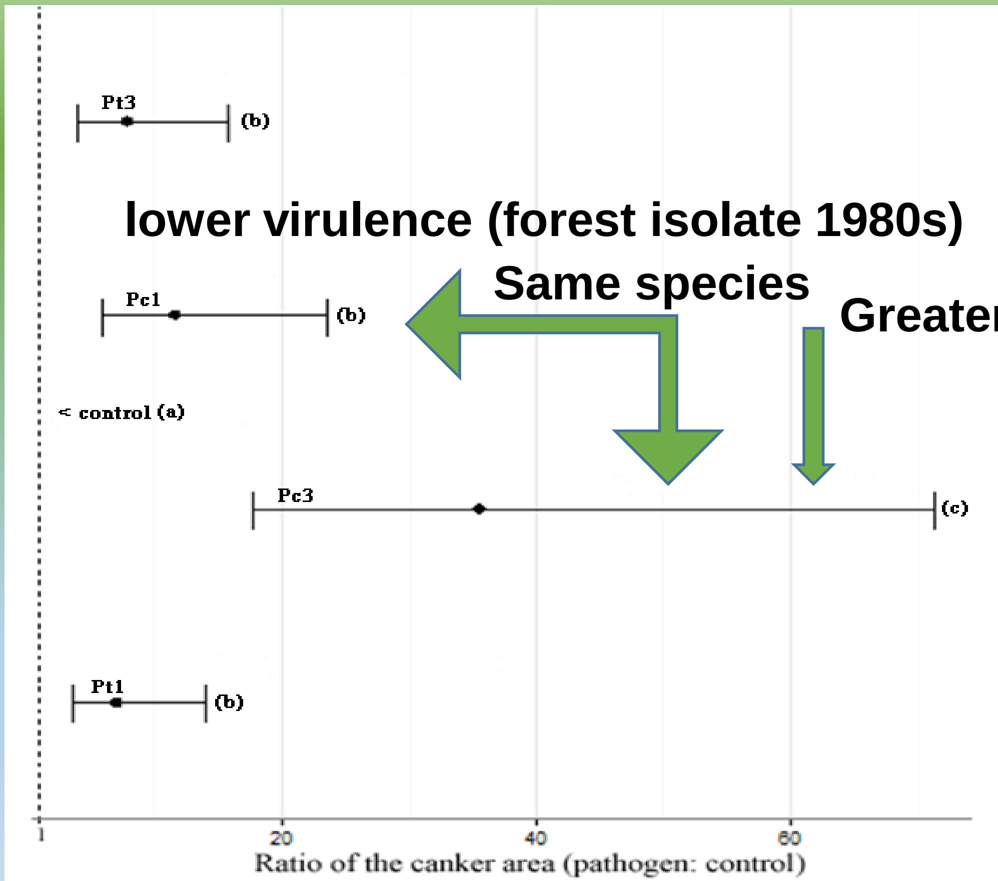
Sims, L.L et al. 2019. *Fungal Biology*

Survived high doses of fungicide, and continued growth suggesting a history of exposure for nurseries and wildlands



Sims, L.L et al. 2019. *Fungal Biology*

Evaluation of virulence & ability to cause disease (koch's postulate successful on four hosts)



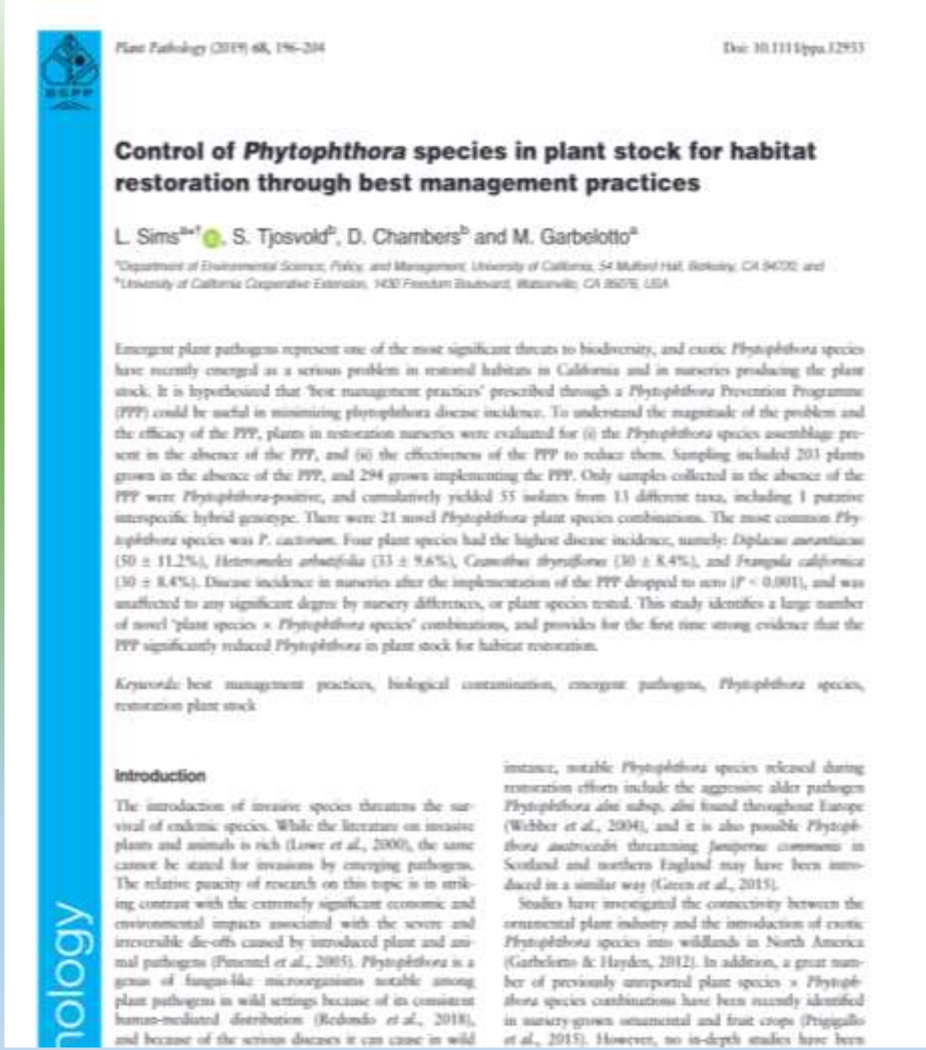
Sims L, Garbelotto M. 2018. *Forest Pathology.*

Conclusions:

- Multiple lines of evidence indicating introduction of exotic Phytophthoras in wildlands is happening through the use of infected plant stock grown in infested nurseries.
- Introduced strains may be more aggressive because ofv. Life history in artificial environments
- Further spread of any exotic *Phytophthora* species should be limited through monitoring efforts, the use of mitigation strategies, and by employing pathogen-free plant stock for restoration

Not all news are bad

- Please read BMPs in Plant Pathology Paper. One of the first studies completed using actual plant stock in production cycles
- Best management practices **work** when applied as a *Phytophthora* Prevention Program in just one year
- Focus was in nursery sanitation, water management and soil management



Plant Pathology (2019) 68, 196–204 Doi: 10.1111/ppa.12933

Control of *Phytophthora* species in plant stock for habitat restoration through best management practices

L. Sims[a]*, S. Tjosvold[b], D. Chambers[b] and M. Garbelotto[a]

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Emergent plant pathogens represent one of the most significant threats to biodiversity, and exotic *Phytophthora* species have recently emerged as a serious problem in restored habitats in California and in nurseries producing the plant stock. It is hypothesized that 'best management practices' prescribed through a *Phytophthora* Prevention Programme (PPP) could be useful in minimizing phytophthora disease incidence. To understand the magnitude of the problem and the efficacy of the PPP, plants in restoration nurseries were evaluated for (i) the *Phytophthora* species assemblage present in the absence of the PPP, and (ii) the effectiveness of the PPP to reduce them. Sampling included 203 plants grown in the absence of the PPP, and 294 grown implementing the PPP. Only samples collected in the absence of the PPP were *Phytophthora*-positive, and cumulatively yielded 55 isolates from 13 different taxa, including 1 putative interspecific hybrid genotype. There were 21 novel *Phytophthora*-plant species combinations. The most common *Phytophthora* species was *P. cactorum*. Four plant species had the highest disease incidence, namely: *Diplacus aurantiacus* (50 ± 11.2%), *Heteromeles arbutifolia* (33 ± 9.6%), *Ceanothus thyrsiflorus* (30 ± 8.4%), and *Frangula californica* (30 ± 8.4%). Disease incidence in nurseries after the implementation of the PPP dropped to zero ($P < 0.001$), and was unaffected to any significant degree by nursery differences, or plant species tested. This study identifies a large number of novel 'plant species × *Phytophthora* species' combinations, and provides for the first time strong evidence that the PPP significantly reduced *Phytophthora* in plant stock for habitat restoration.

Keywords: best management practices, biological contamination, emergent pathogens, *Phytophthora* species, restoration plant stock

Introduction

The introduction of invasive species threatens the survival of endemic species. While the literature on invasive plants and animals is rich (Lowe *et al.*, 2000), the same cannot be stated for invasions by emerging pathogens. The relative paucity of research on this topic is in striking contrast with the extremely significant economic and environmental impacts associated with the severe and irreversible die-offs caused by introduced plant and animal pathogens (Pimentel *et al.*, 2005). *Phytophthora* is a genus of fungus-like microorganisms notable among plant pathogens in wild settings because of its consistent human-mediated distribution (Redondo *et al.*, 2018), and because of the serious diseases it can cause in wild

instance, notable *Phytophthora* species released during restoration efforts include the aggressive alder pathogen *Phytophthora alni* subsp. *alni* found throughout Europe (Webber *et al.*, 2004), and it is also possible *Phytophthora austrocedri* threatening *Juniperus communis* in Scotland and northern England may have been introduced in a similar way (Green *et al.*, 2015).

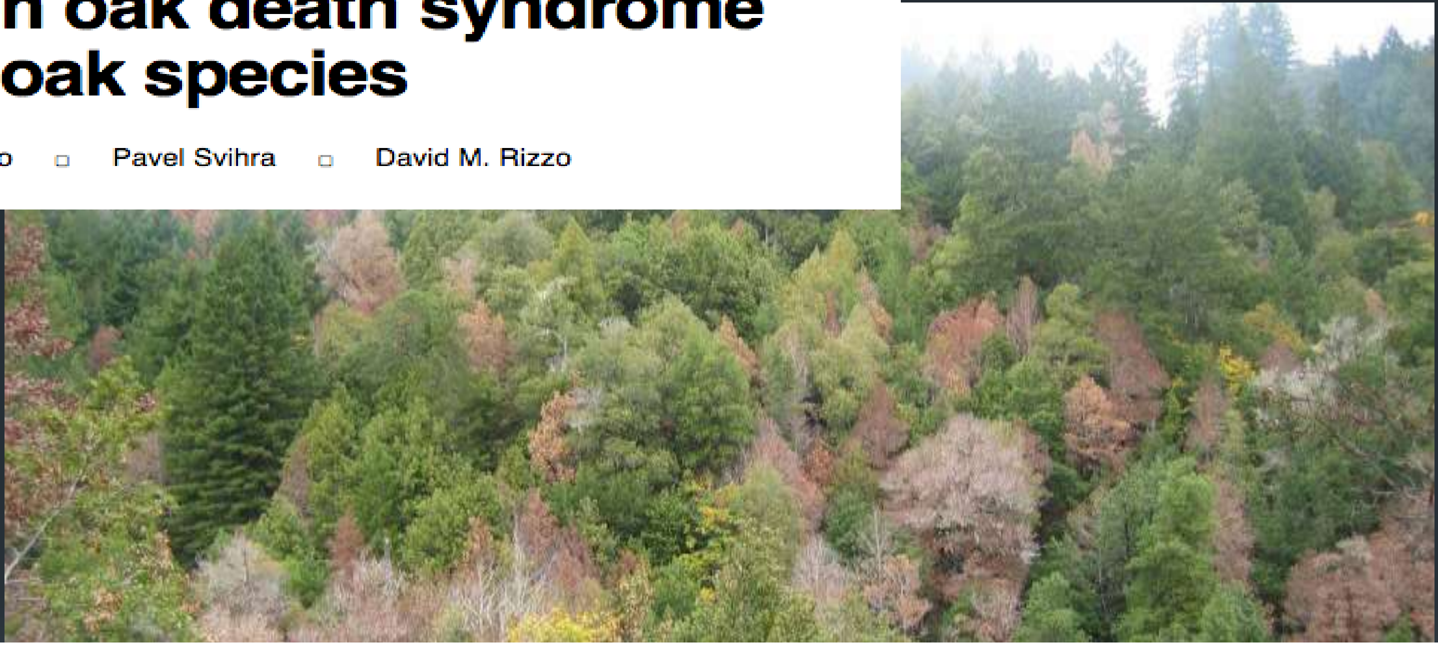
Studies have investigated the connectivity between the ornamental plant industry and the introduction of exotic *Phytophthora* species into wildlands in North America (Garbelotto & Hayden, 2012). In addition, a great number of previously unreported plant species × *Phytophthora* species combinations have been recently identified in nursery-grown ornamental and fruit crops (Prigigallo *et al.*, 2015). However, no in-depth studies have been

New pests and plant diseases

Sudden oak death syndrome fells 3 oak species

Matteo Garbelotto ▫ Pavel Svihra ▫ David M. Rizzo

2000 AD



Phytophthora ramorum as the Cause of Extensive Mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California

2001

D. M. Rizzo, Department of Plant Pathology, University of California, Davis 95616; **M. Garbelotto,** Department of Environmental Science, Policy and Management, Ecosystem Science Division, University of California, Berkeley 94720; **J. M. Davidson** and **G. W. Slaughter,** Department of Plant Pathology, University of California, Davis; and **S. T. Koike,** University of California Cooperative Extension, 1432 Abbott Street, Salinas, CA 93901

Why do we care about Sudden Oak Death?

- Over 50 million trees already lost

- Ecological effects:

--forests look different
--wildlife impacts

- Social effects:

--hazard trees
--fire risk
--economic costs
--emotional impacts

- Ongoing threat:

--30% of susceptible forest affected so far

Ecological Impacts

- There are about 110 species of birds which breed in California's oak woodlands. Another 60 or so species use oak woodlands outside the breeding season.
- 105 mammal species.
- 58 amphibians and reptiles
- An estimated 5,000 species of insects.
- An unknown number of microbes.
- Wide variety of other trees, shrubs and flowering plants which co-exist with oak woodlands.

Phytophthora ramorum

- 4 different subspecies (lineages)
- Origin in SouthEast Asia (China/Vietnam)
- Ornamental trade, worldwide
- Hundreds of host species
- Different diseases: from mild to lethal depending on host

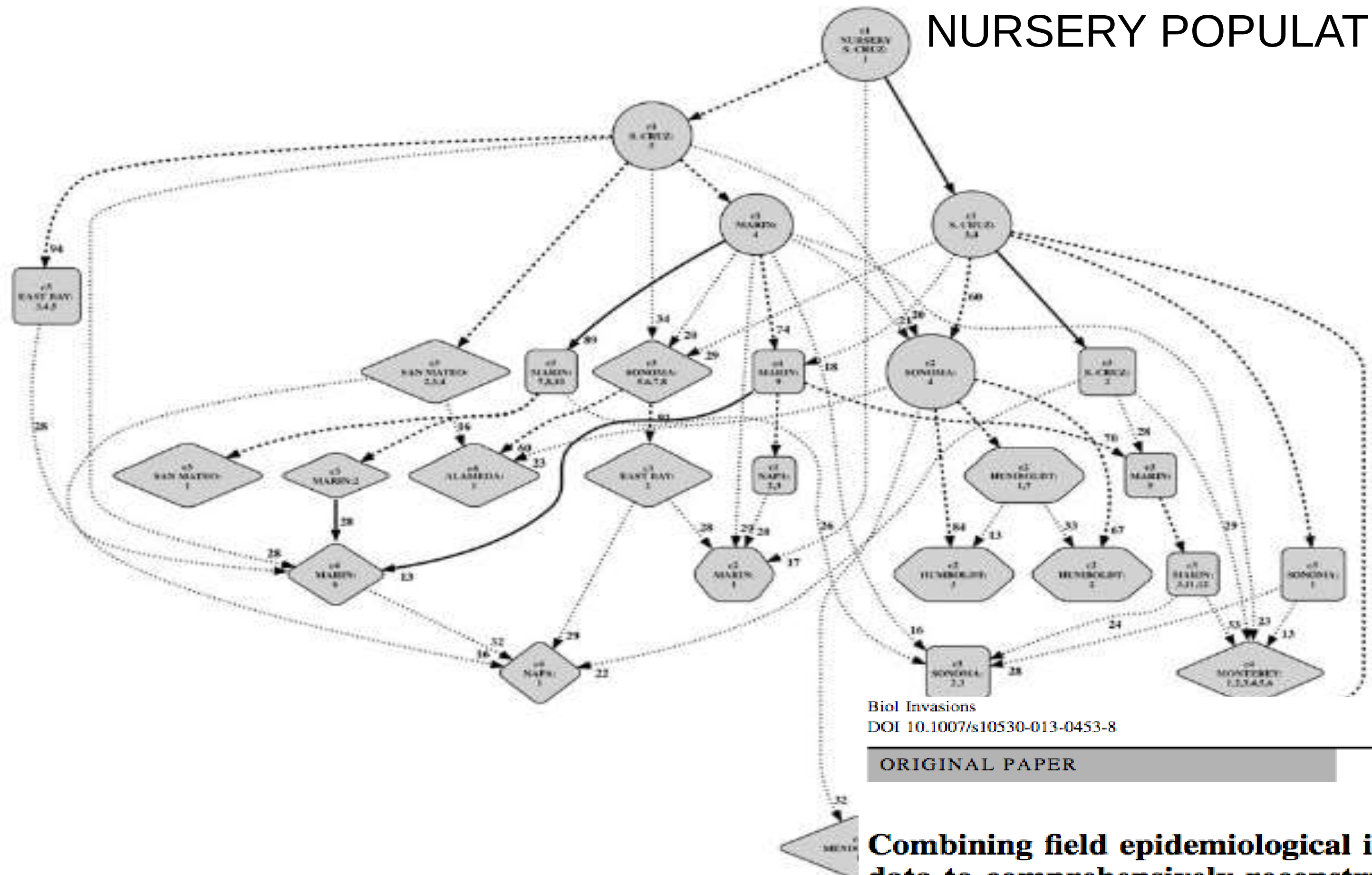


Fig. 5 MIGRATE-N model-choice based reconstruction of the possible colonization routes of *P. ramorum* during the Californian SOD epidemic. Population names are indicated at nodes along with genetic cluster ("clade") membership. *Nodes shapes* correspond approximately to time since infestation: *circle* 20+ years; *square* 15–20 years; *diamond* 10–15 years;

hexagon ≪1
populations ‹
bold-dashed
50 % of the
Numbers ne›
migration rou

Biol Invasions
DOI 10.1007/s10530-013-0453-8

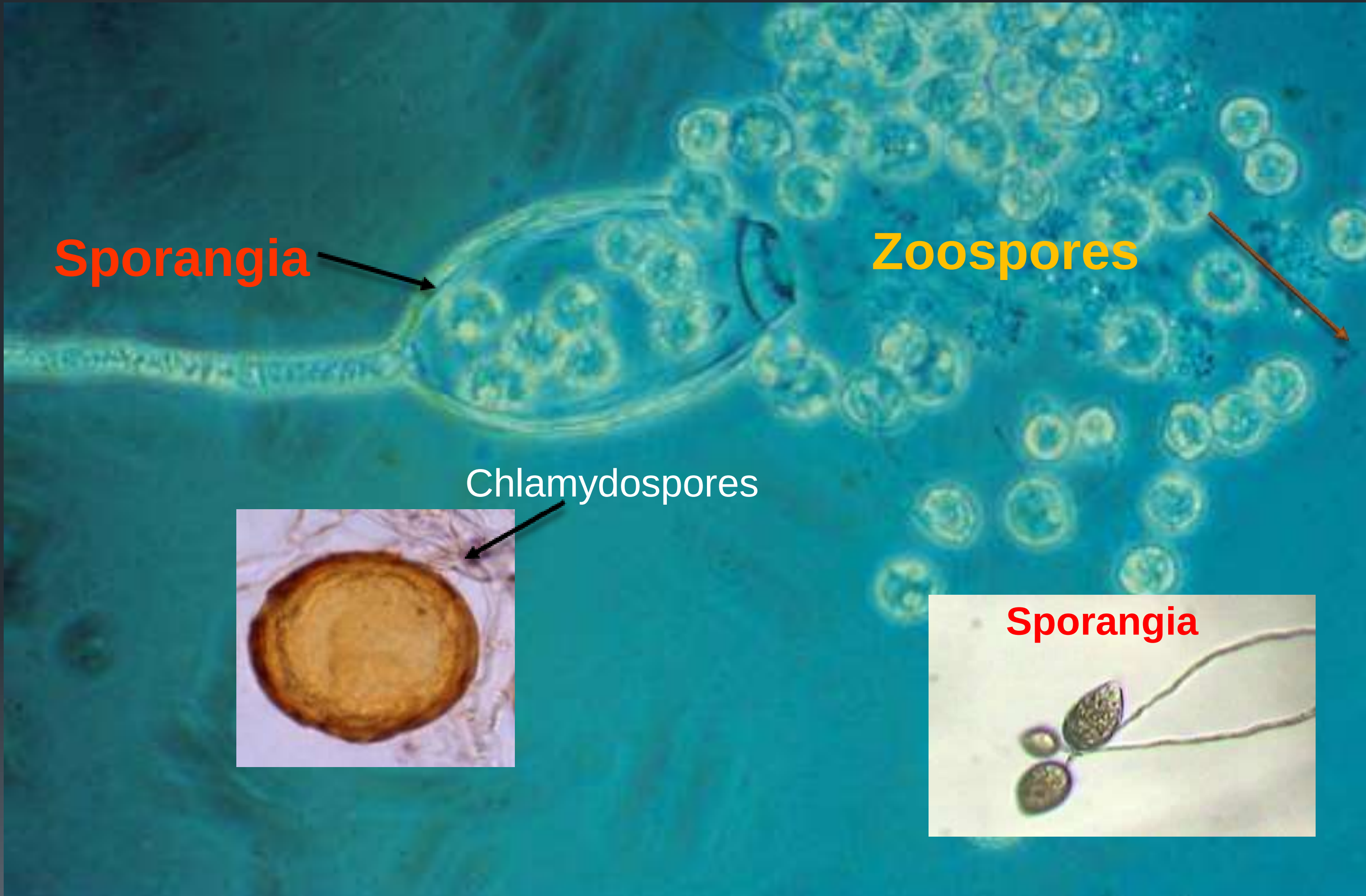
ORIGINAL PAPER

Combining field epidemiological information and genetic data to comprehensively reconstruct the invasion history and the microevolution of the sudden oak death agent *Phytophthora ramorum* (Stramenopila: Oomycetes) in California

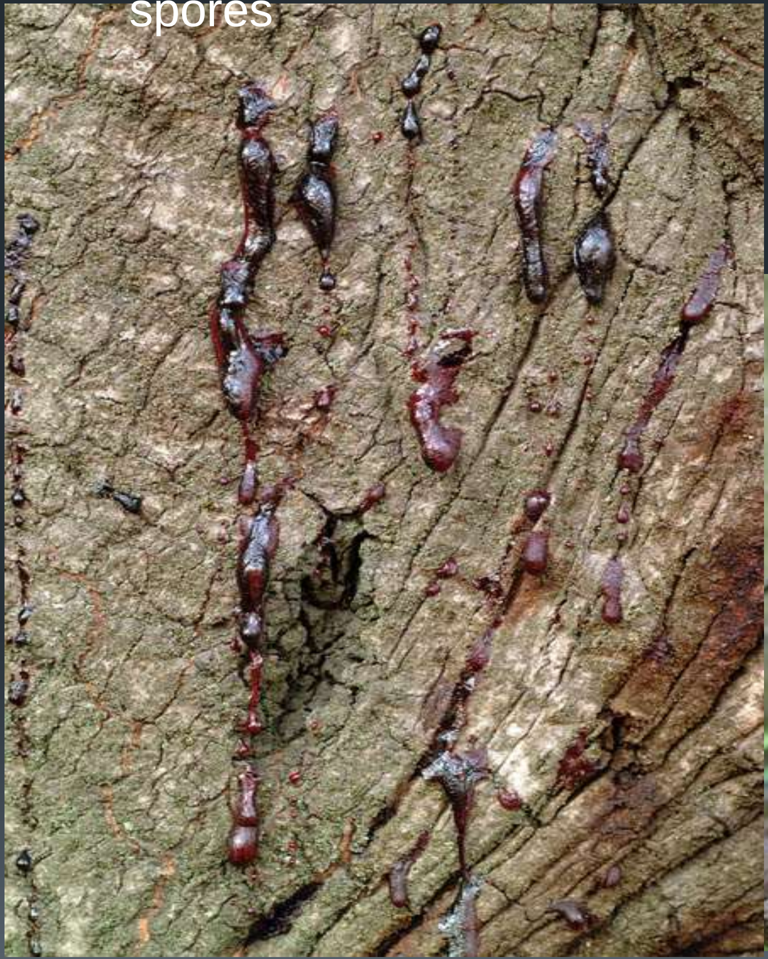
Peter J. P. Croucher · Silvia Mascheretti ·
Matteo Garbelotto

Aerial species

- First discovered for temperate forests: characterized by deciduous sporangia
- Splash dispersed: sporangia do not dry
- True aerial will naturally infect aerial parts without need for root infections or transmission by tools
- Ability to rest in soil with resting structures is not lost!!, but no epidemiological relevance of soil or water infestations

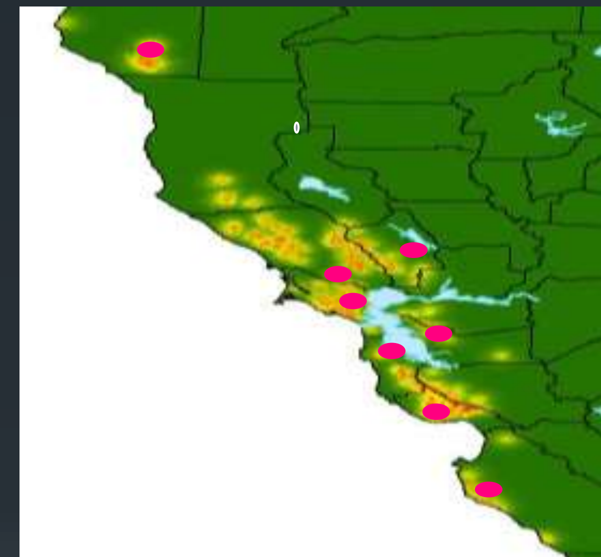

Sporangia
Zoospores
Chlamydospores
Sporangia

Aerial stem cankers on oak spp. and tanoaks: deadly but not infectious, e.g. stem lesions do not produce significant number of spores



Girdling aerial 'cankers' removed from roots

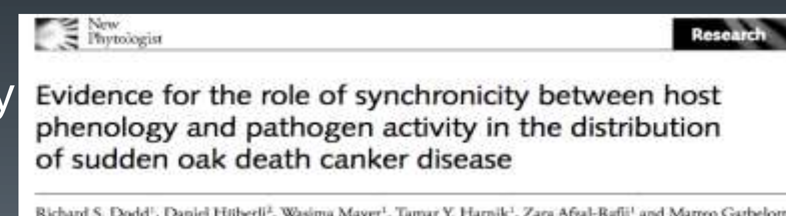
P. ramorum introduced at least 12 times in CA (Croucher et al. 2013). Multiple introductions and not ability to move far explain distribution of disease



Pathogen is exotic:

1 -native flora has limited resistance, but additionally

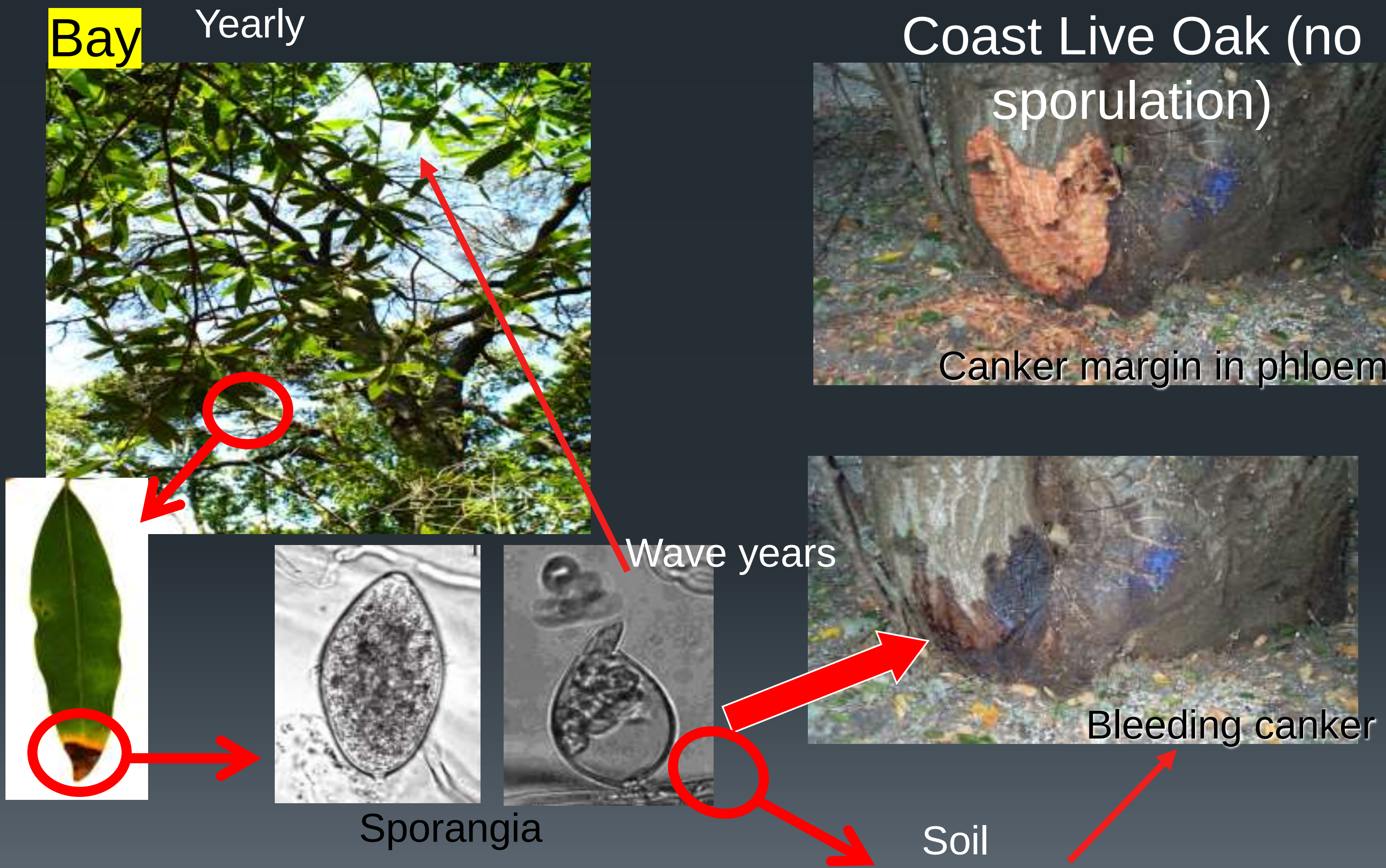
2- synchronicity between sporulation and host susceptibility (perfect ecological match)


New Phytologist — Research

Evidence for the role of synchronicity between host phenology and pathogen activity in the distribution of sudden oak death canker disease

Richard S. Dodd[1], Daniel Hüberli[2], Wasima Mayer[1], Tamar Y. Harnik[1], Zara Afzal-Rafii[1] and Matteo Garbelotto[1]

Bay/Oak association



Populations of *P. ramorum* can be differentiated in at least 2 ways:

- Aerial (plant) vs. soil vs. water communities
- Transmissive vs. dead-end hosts

For. Path.

doi: 10.1111/j.1439-0329.2011.00715.x

Phytophthora ramorum is a generalist plant pathogen with differences in virulence between isolates from infectious and dead-end hosts

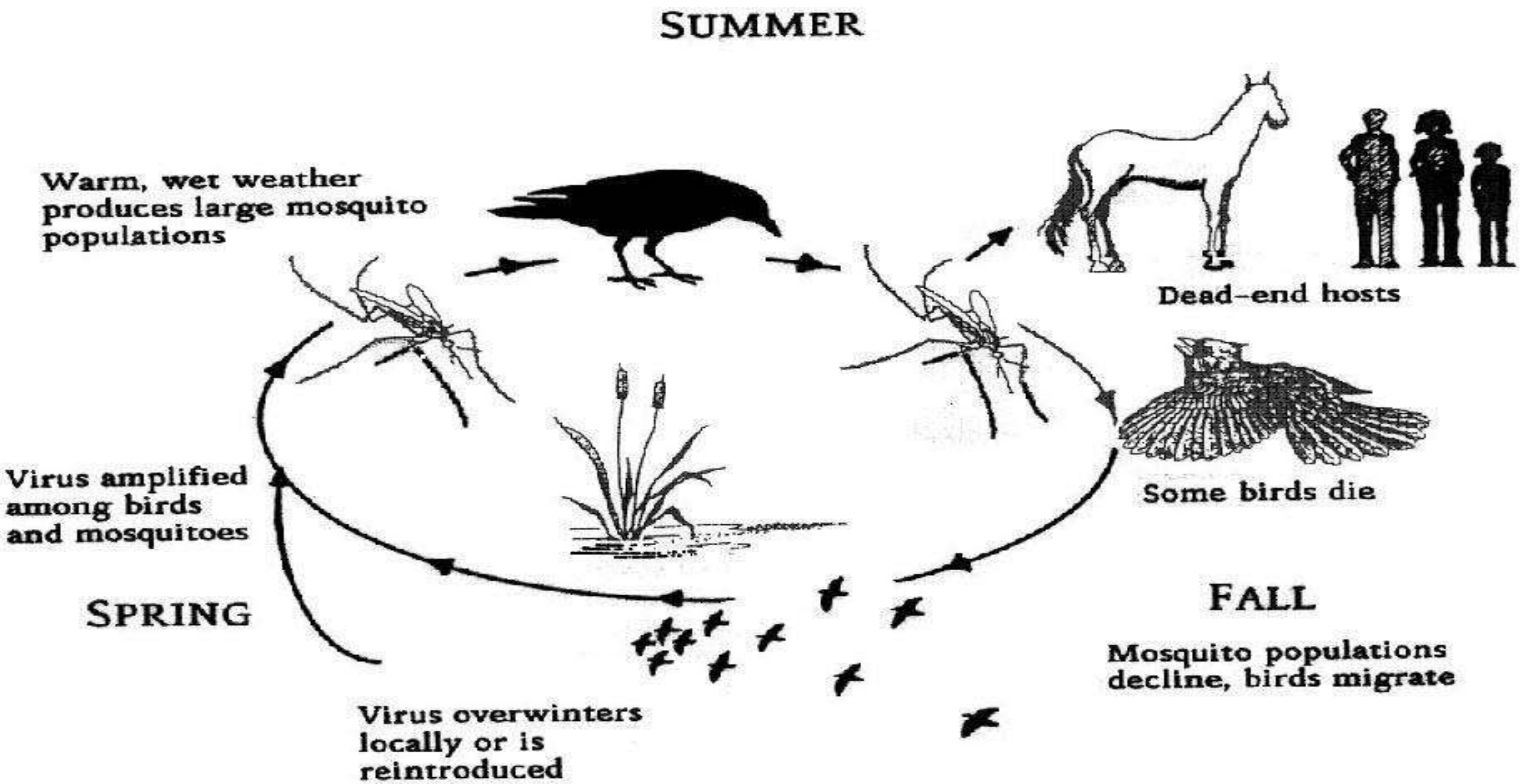
By D. Hüberli* and M. Garbelotto[1]

OPEN ACCESS Freely available online PLoS one

Phenotypic Diversification Is Associated with Host-Induced Transposon Derepression in the Sudden Oak Death Pathogen *Phytophthora ramorum*

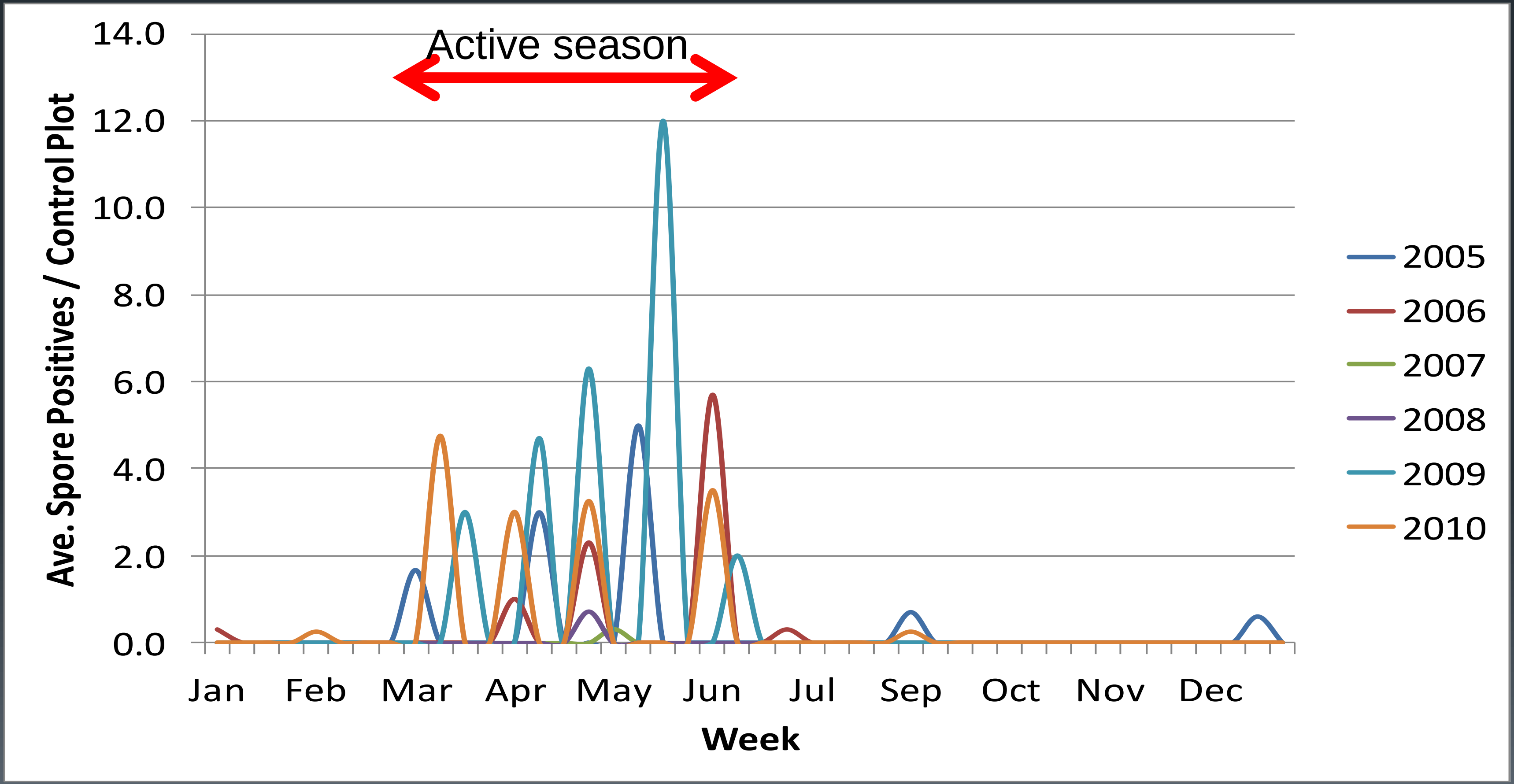
Takao Kasuga[1], Melina Kozanitas[2], Mai Bui[1], Daniel Hüberli[2¤], David M. Rizzo[3], Matteo Garbelotto[2*]

Life Cycle of the West Nile Virus



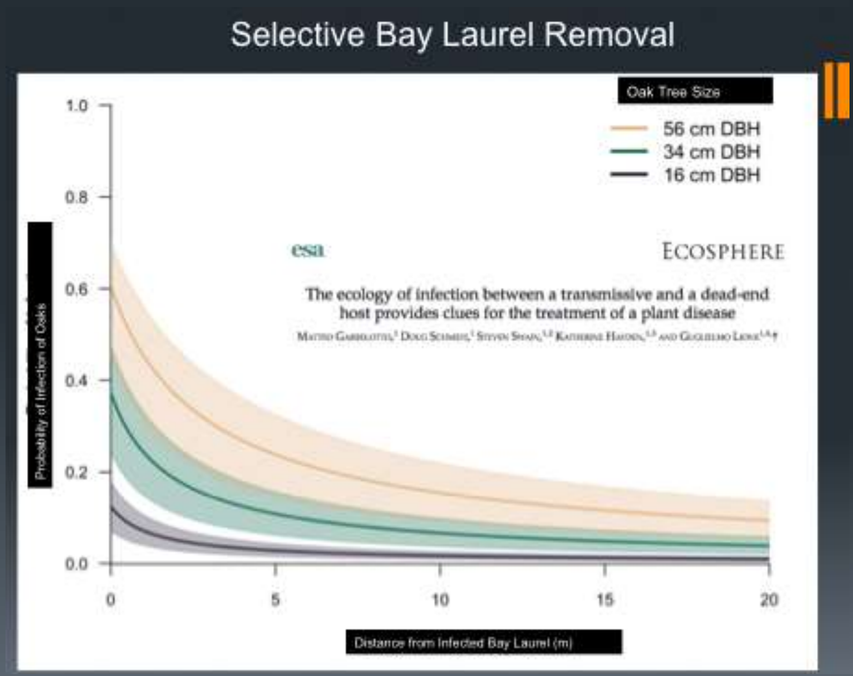
SOD spore catches in water: mid-April to mid-June is consistent

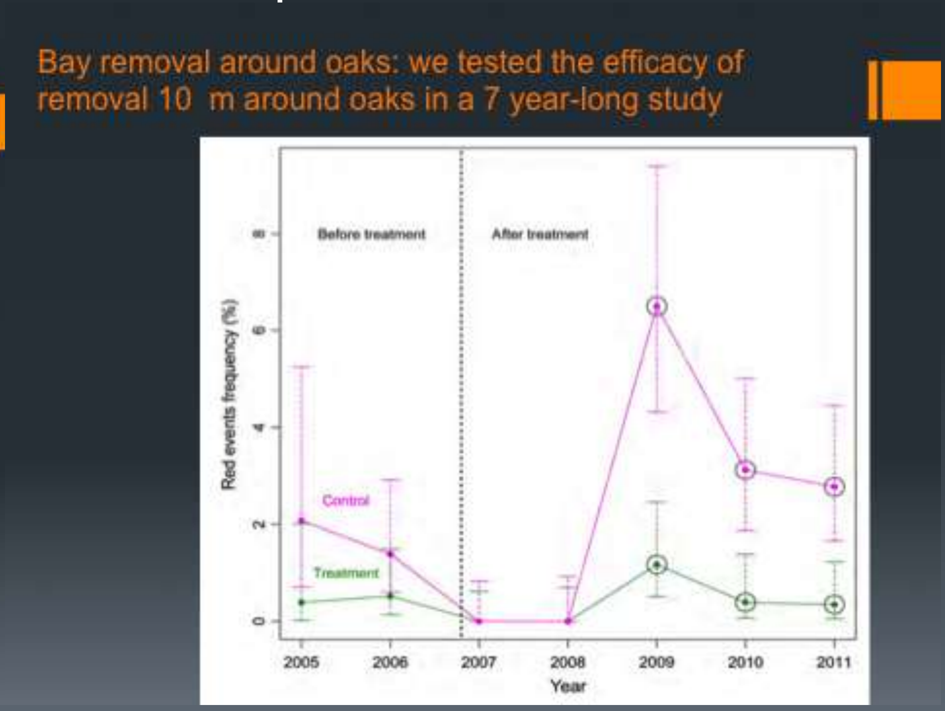
Average Temperatures in Plots



For infection to occur, infectious host and dead-end host need to be near each other

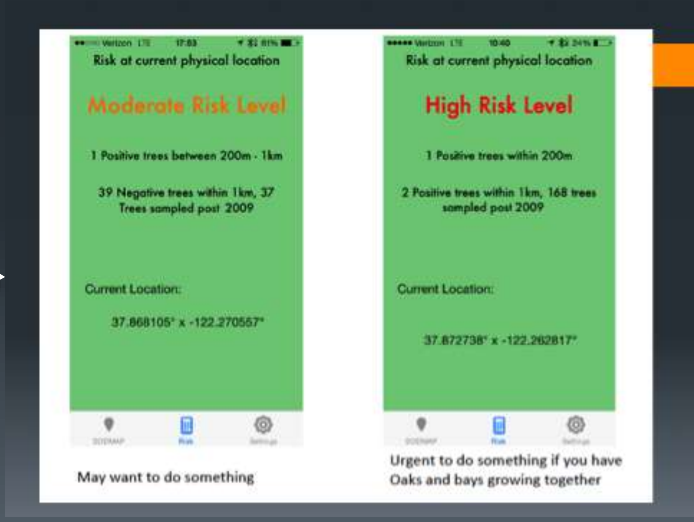
- "Social distancing" works for forest diseases too. How?
- Oaks need to be within 10 m from laurel bays to be infected
- Take out bays 10 m around oaks to be protected. It works!





Like during the Covid-19 pandemic, how do we know who is infected? Resources are scarce and how do we share the data?

500 volunteers per year generate data used by millions
(red dots are laurels with SOD, green dots are healthy laurels!)



Phytophthora cinnamomi (Pc)

- One of the first pathogens to be transported and introduced globally in the Anthropocene from Papua NewGuinea and or Sumatra
- One of the 100 most invasive organisms, relevant both in agriculture and in natural ecosystems around the world
- In North American wildlands: older introduction on the East coast, affecting pines and chestnuts

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

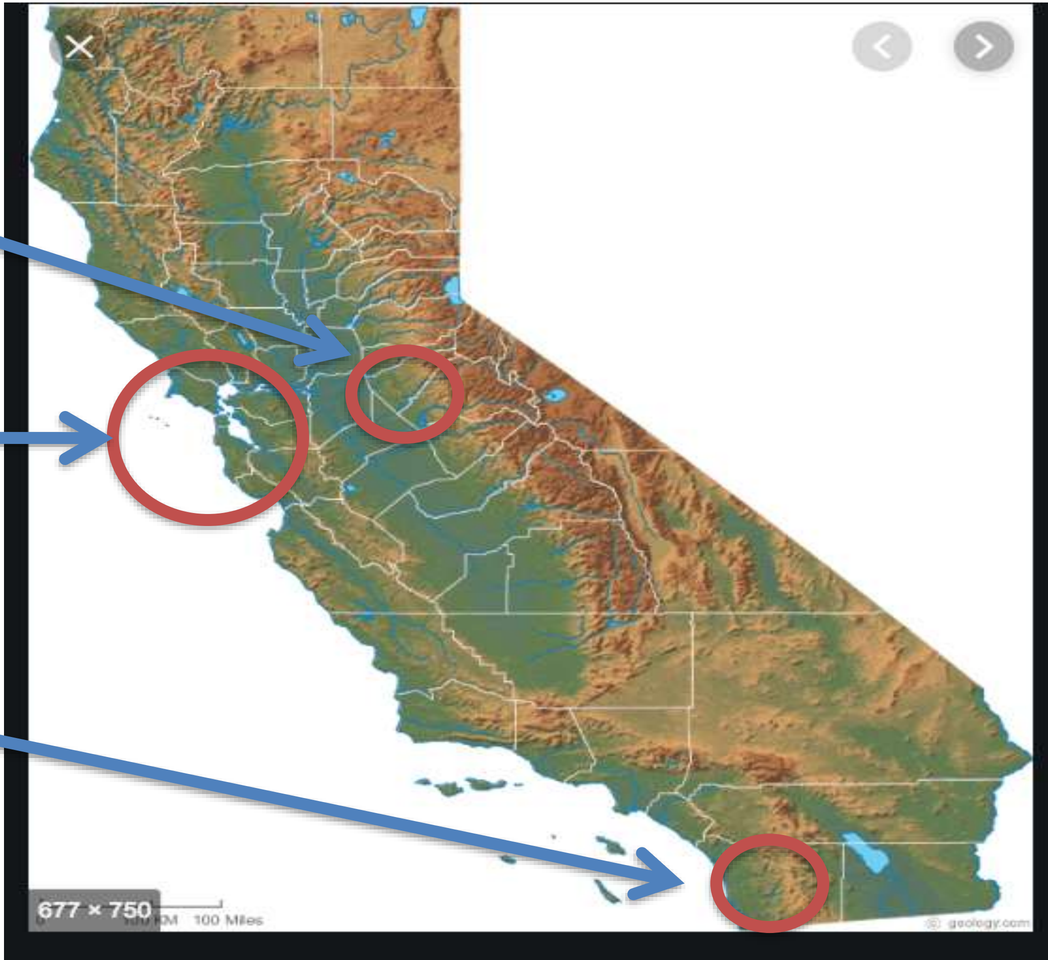
Pc killing drought stressed oaks in So-cal (27% infected of 474 tested) in areas next Avocado orchards

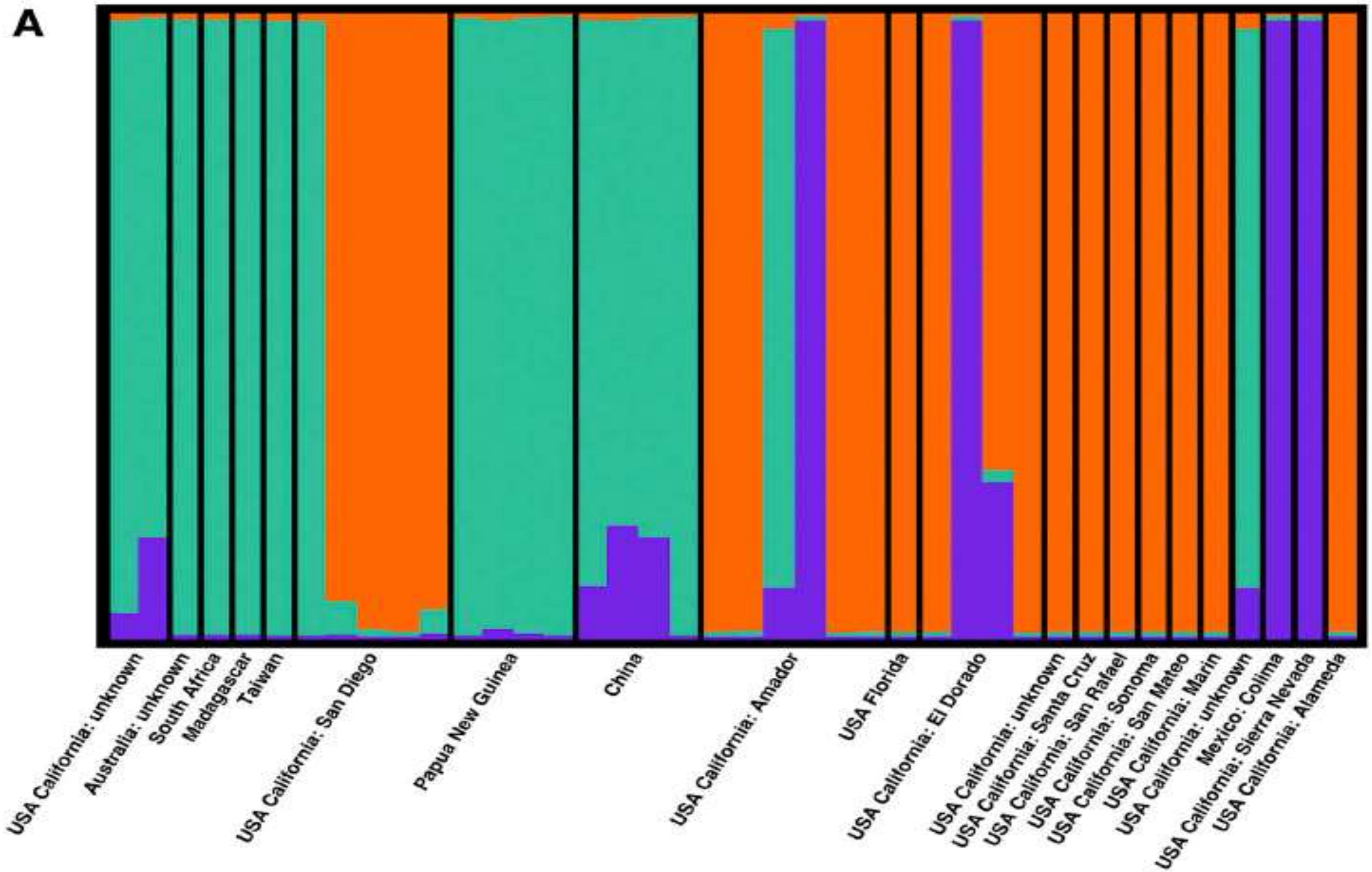
Geographic separation of CA outbreaks

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

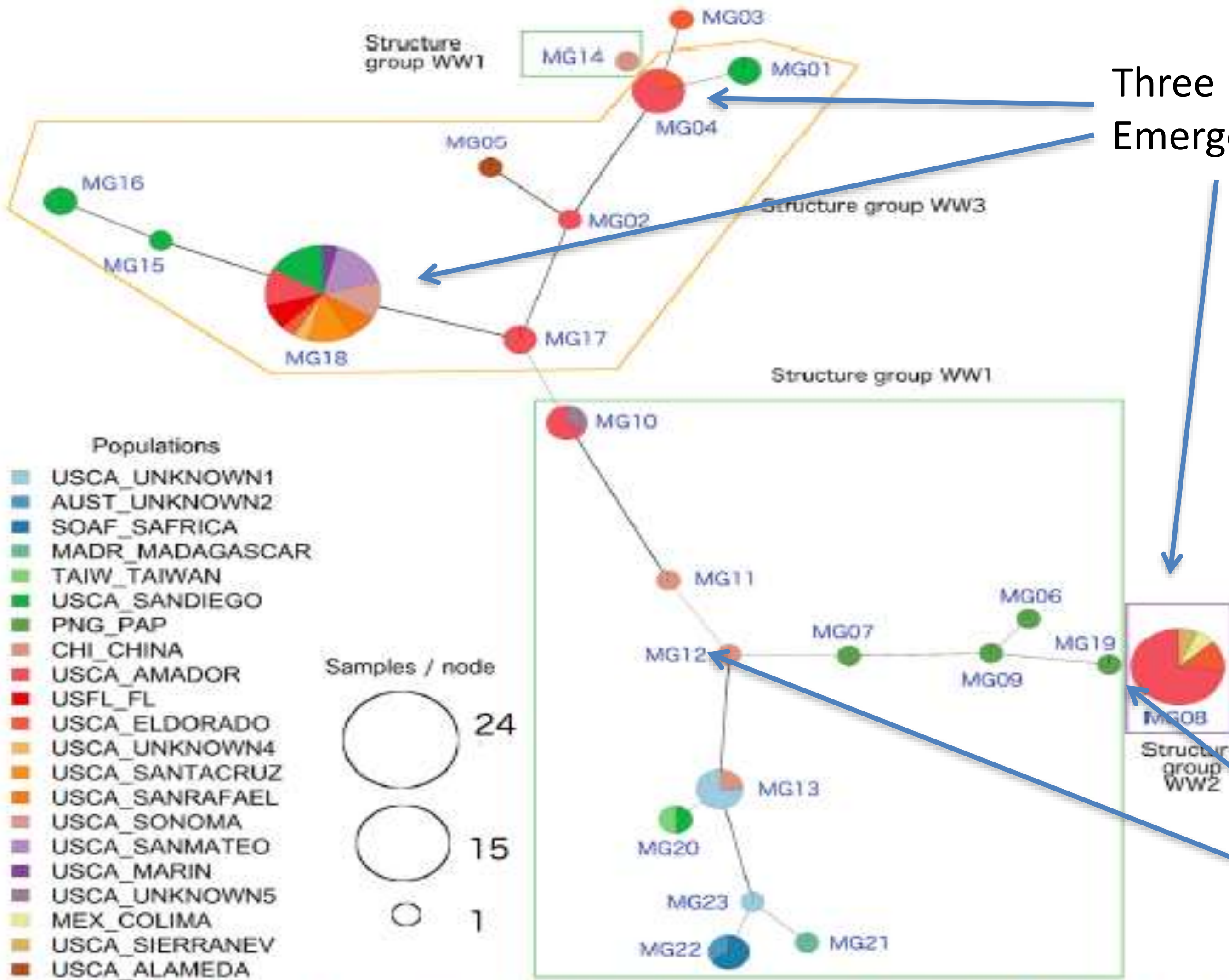
2- Pacific madrone, bay laurels and manzanitas in hotspots of disease around the SF Bay Area

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





Green= www1 ; Orange = www3; Purple= www2



Three MLGs are Emergent or invasive

Switch To A2

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.

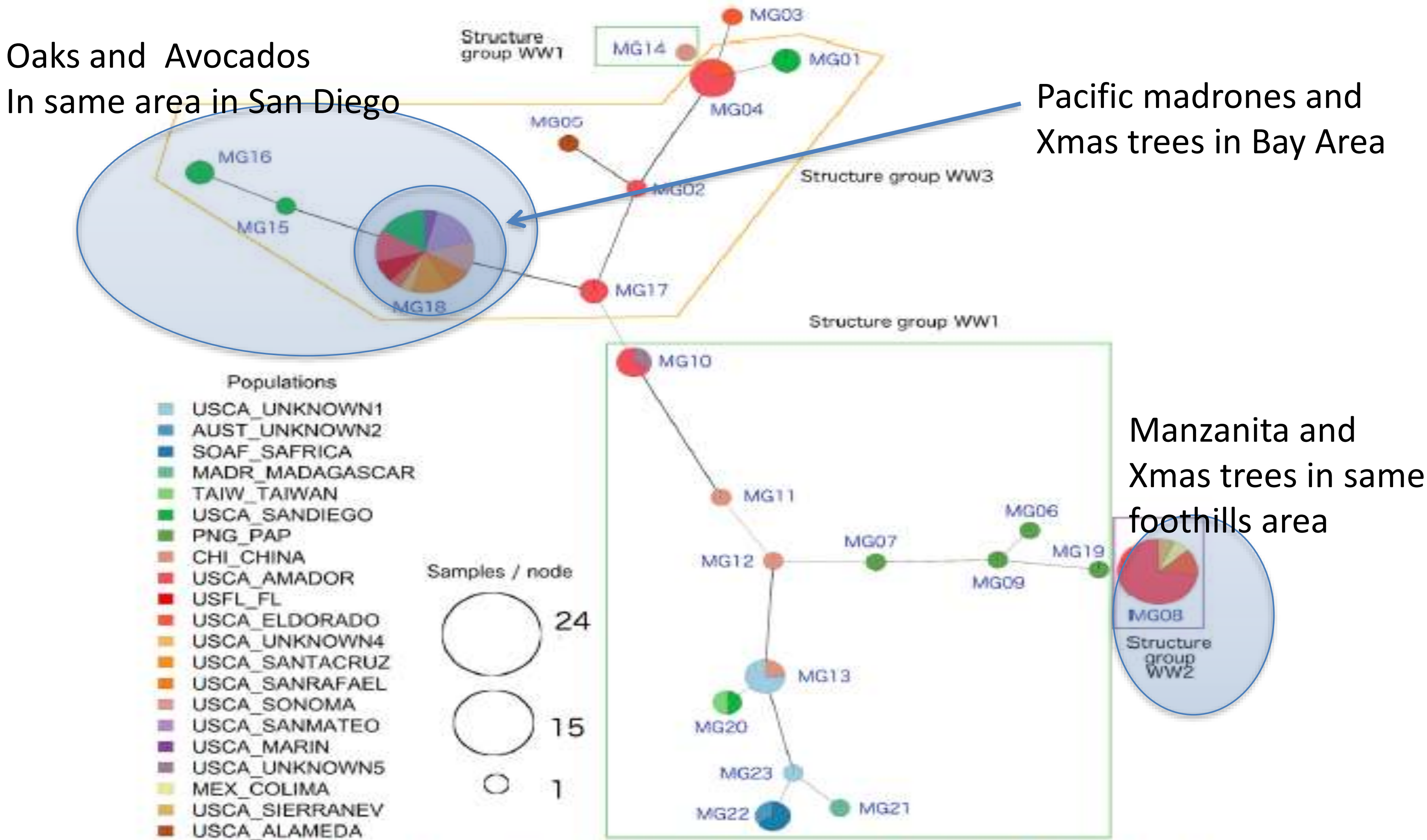
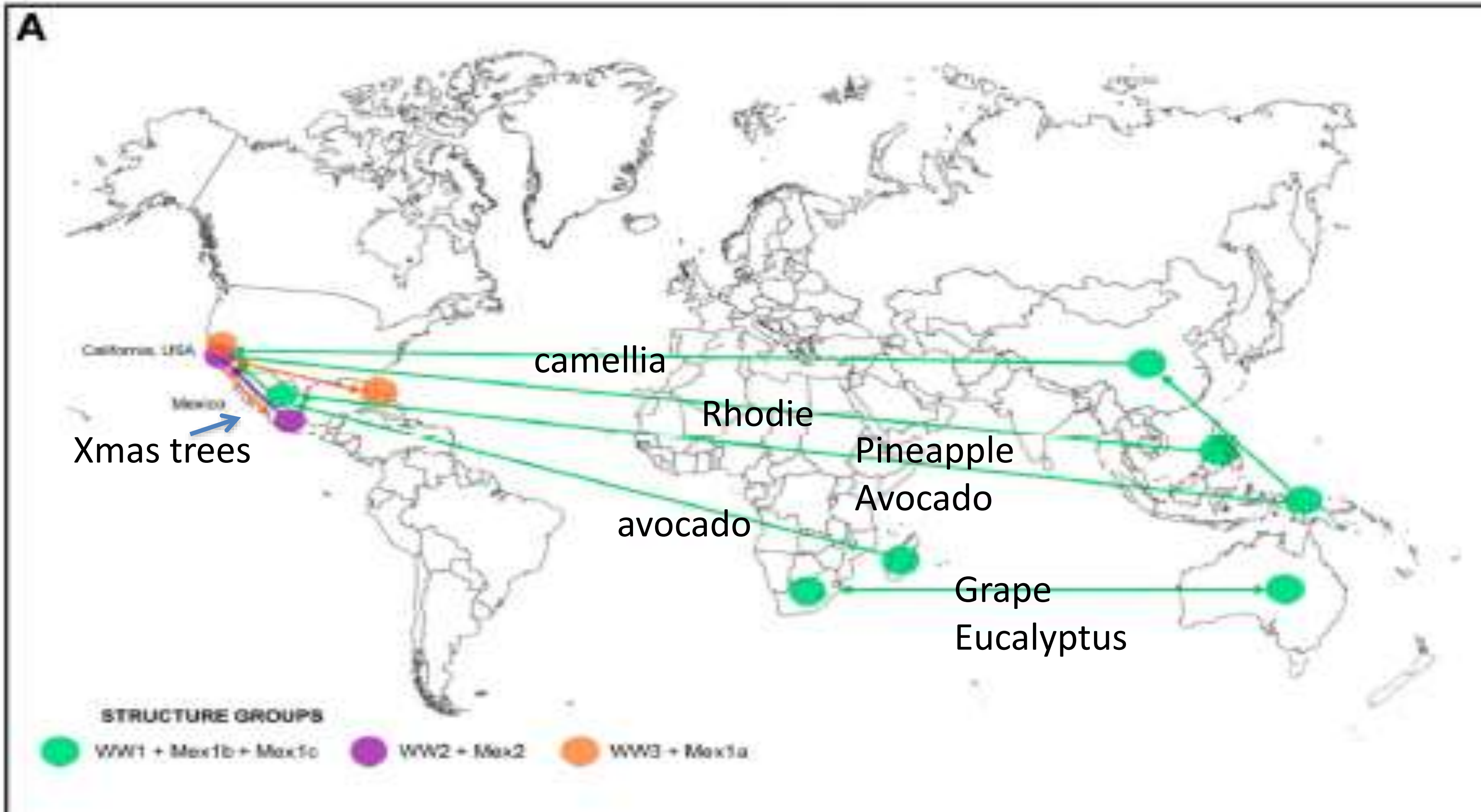
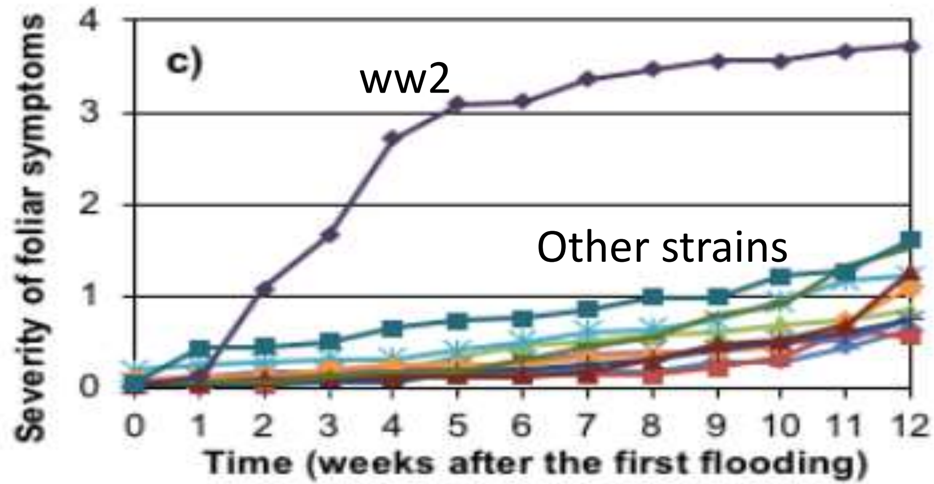


Fig. 3. Minimum spanning network based on the matrix of pairwise Bruvo genetic distances visualizing relatedness among multilocus genotypes (MGs) in the worldwide/California analysis.

Same MLG in different parts of the world. WW1 ancestral



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]

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

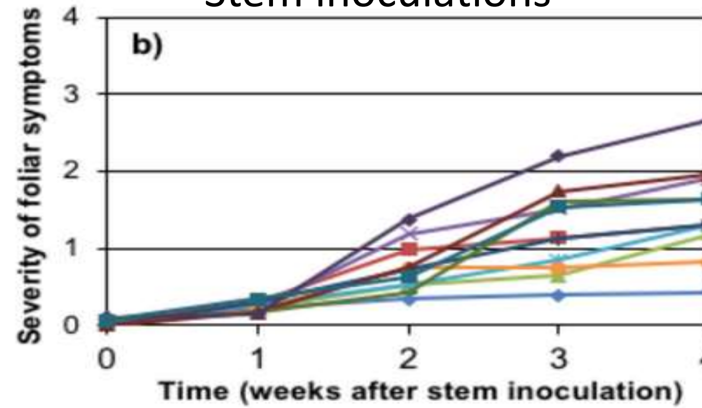
PSA= Percentage Stem affected

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

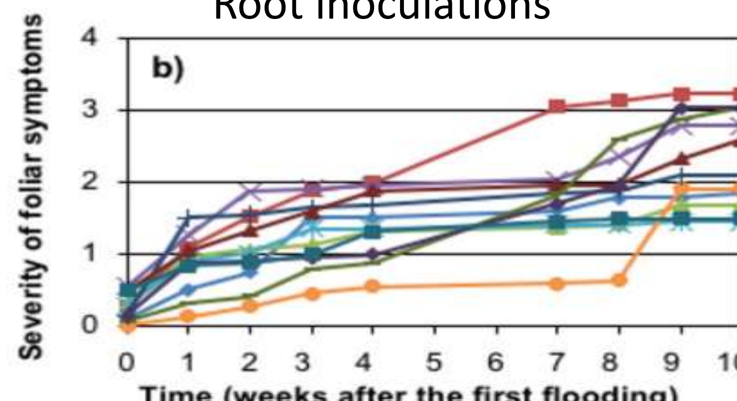
Is there significant host x pathogen genotype interaction (Douglas-fir)

- 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?**

Prevent further spread

Ensure trade is pathogen-free (HUGE TASK)

- Train people to identify symptoms at ports of entry
- Use robust sampling for asymptomatic plants, including pooling of samples
- Use molecular tests (*P. ramorum* was the first pathogen regulated using DNA!)
- Use new approaches:
    - Test water run off
    - Use dogs

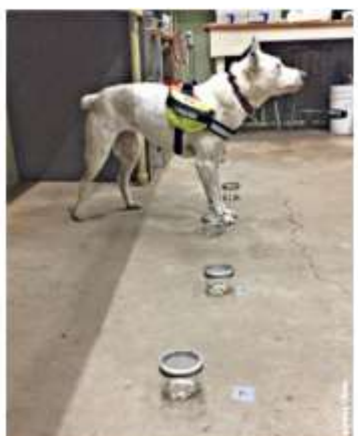
RESEARCH ARTICLE

Three new *Phytophthora* detection methods, including training dogs to sniff out the pathogen, prove reliable

A scent detection dog identified *Phytophthora* in media with a 100% accuracy; two other simple and cost-effective methods detected the pathogen with great confidence directly from plants.

by Tedmund J. Swiecki, Matt Quinn, Laura Sims, Elizabeth Bernhardt, Lauralea Oliver, Tina Popenuck and Matteo Garbelotto

Imprinting the odor of *Phytophthora* using a container drill.



Detection dog displaying anticipatory, or alert, behavior at the one container that contained *Phytophthora*.

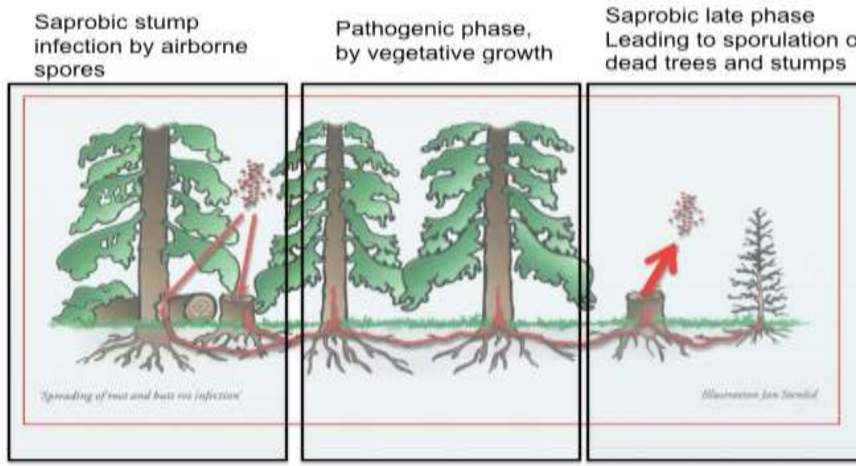
Heterobasidion root rot (a fungus)

- Most damaging root disease of conifers in the world,
but in primary forest its incidence is rare and its effects beneficial.

- In Western USA and in California there are two species.

- Each species is host specific
  - *Heterobasidion irregulare* kills pines, junipers, incense cedars and some angiosperms
  - *Heterobasdion occidentale* infects true firs, hemlocks, sequoias and Douglas-firs. It can be endophytic or pathogenic on these hosts

Logging greatly increases incidence of *H. irregulare* because of stumps



Incidence of *H. occidentale* increases with increasing true fir populations. Fire exclusion has resulted in a huge expansion of true firs, leading to a huge increase in populations of *H. occidentale.*

Where true fir has become invasive in pine stands both *Heterobasidion* species present and are hybridizing on stumps

Sequoias infected by *H. occidentale*

1998

Use of Taxon-Specific Competitive-Priming PCR to Study Host Specificity, Hybridization, and Intergroup Gene Flow in Intersterility Groups of *Heterobasidion annosum*

Matteo Garbelotto, Alice Ratcliff, Thomas D. Bruns, Fields W. Cobb, and William J. Otrosina

2008

Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of interspecific horizontal genetic transfer and of human-mediated, long-range dispersal

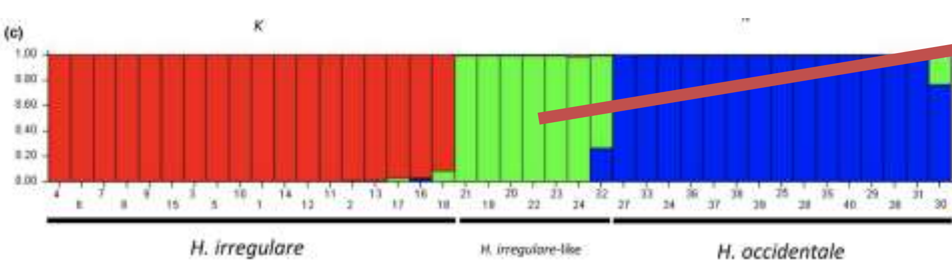
R.E. Linzer[a], W.J. Otrosina[b], P. Gonthier[c], J. Bruhn[d], G. Laflamme[e], G. Bussières[f], M. Garbelotto[a,*]

Hybridization and interspecific genic introgression is happening with hard-to-predict outcomes

2018

Molecular analyses identify hybridization-mediated nuclear evolution in newly discovered fungal hybrids

Fabiano Sillo[1] | Paolo Gonthier[1] | Blakey Lockman[2] | Takao Kasuga[3] | Matteo Garbelotto[1,4]



New hybrid species is responsible for Alpine larch mortality In Montana

FIGURE 1 Alpine larches (*Larix lyallii*) in the Bitterroot Mountains (Montana, USA) showing disease symptoms (a) related to the infection by *Heterobasidion* spp. In (b), *Heterobasidion* spp. fruit body (indicated by a white arrow) developed at the base of a fallen tree

Mortality of native trees adapted to mesic environments that either have been planted offsite (redwoods) or have become invasive because of fire exclusion (California bay laurels)

Mortality of blue oaks and Grey pines in harsh Sierra foothills

Large scale mortality of planted exotic acacias and eucalyptus

Mortality of manzanitas growing in dry and hot sites

Investigating the potential fungal involvement in the widespread decline (dieback) of trees in Northern California

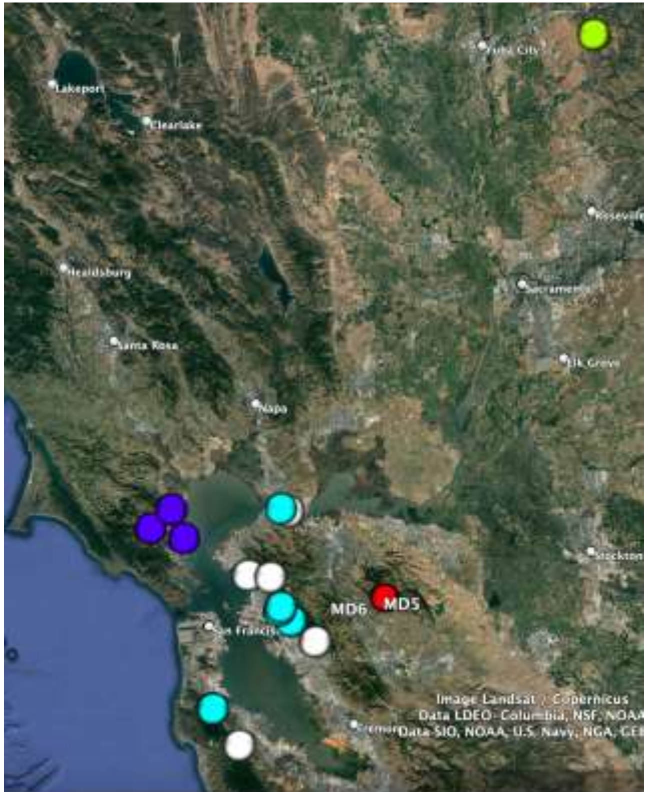
Matteo Garbelotto
U.C. Berkeley


Ines Marques
Tina Popenuck
Doug Schmidt
Funding by:
SFPUC
East Bay Regional Parks
US FOREST SERVICE

Objectives: Understanding the drivers of the large-scale dieback of exotic and native species observed in Northern California

- What are the general symptoms and or signs (e.g. cankers, wood staining, fungal structures, etc.) associated with the observed dieback?
- Are there fungi that, because isolated from all study locations and given their known biology, may be playing a primary role
- Are there other fungi that may be playing a role, and are they site-specific or are they shared among sites?
- Are the fungi involved native or exotic?

STUDY SITES



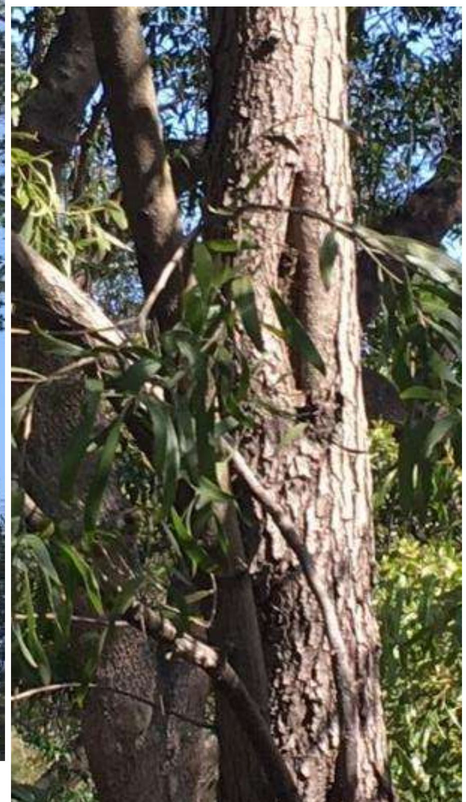
- 1-16 trees per site were felled and dissected
- Only symptomatic tissue was collected
- Culturing on six different growth media
- Soil was collected under each tree
- Soil Baiting using three different bait types

Key to map:

white= Blue gums
blue= Blackwood acacias
green= Blue oaks
red= Manzanitas (2 spp.)
purple= Ca Bay Laurels

Leona Heights, Oakland, April 2021. Acacia dieback

Cankers on acacia – associated with dieback

Acacia Dieback Project

Figure 1. Examples of dieback in eucalyptus.

Figure 2. Examples of collected material.

Only consistent symptom observed
In Eucalyptus was leaf blight and
twig/branch lesions

Occasionally stem or branch
cankers , heartrot, etc.

Manzanitas
Mount Diablo

A dying blue oak (foreground) in Butte County with healthy blue oaks in the background.

Results: over 300 isolates were obtained
Major players (isolated frequently)

Eucalyptus

- A total of 124 plant samples was collected from 24 trees
- The only symptoms that were widespread were foliage browning and twig cankers
- A diverse assemblage of fungi was isolated at each site, however the only ubiquitous fungi were the leaf blight fungi ***Pseudosydowia eucalypti*** *(**Pe**)* and *a **Cladosporium sp**.*

Acacia

- A total of 81 samples was collected from 30 trees
- Stem and branch cankers were visible at each site
- A diverse assemblage of fungi was isolated at each site, however the canker fungi ***Diaporthe foeniculina (Df)*** and ***Dothiorella viticola (Dv)***were present at each site
- ***Umbelopsis rammaniana*** in a single site?

Manzanitas, blue oaks, bays

- A total of 90 samples was collected from 25 trees
- Stem and branch cankers were visible at each site
- A diverse assemblage of fungi was isolated at each site, however ***Neofusicoccum* spp.**, ***Diplodia* spp** and ***Botryosphaeria dothidea*** were dominant

Any sign of alien pathogens?

- Acacia
  - ***Dothiorella moneti*****:** a pathogen of acacias, exclusively reported from Australia. Known latent pathogen with endophytic stage (inside plant but not causing disease), likely to be moved where acacias are moved
  - Greenhouse inoculations showed it to be slightly more aggressive than *D. viticola,* but need to inoculate adult trees to get better information
  - Only found in one site, but site was maybe worst hit

- Eucalyptus
  - ***Pseudsydowia eucalypti***: although unreported in Ca it is an endophyte and a fungus of unknown virulence, unofficialy known tpo be ubiquitous where Eucalyptus grows
  - ***Neofusicoccum eucalyptorum****:* unreported in California, but found inly in one site so not likely to be major player

ALIEN INTRODUCED FUNGI ARE NOT LIKELY TO BE MAJOR PLAYERS OF MORTALITY OBSERVED IN BOTH ACACIAS AND EUCALYPTUS

Diaporthe foeniculina and *Dothiorella viticola* are either native or long naturalized, but in one site (the worst hit) we also isolated the exotic *D. moneti*, only reported from acacias in Australia

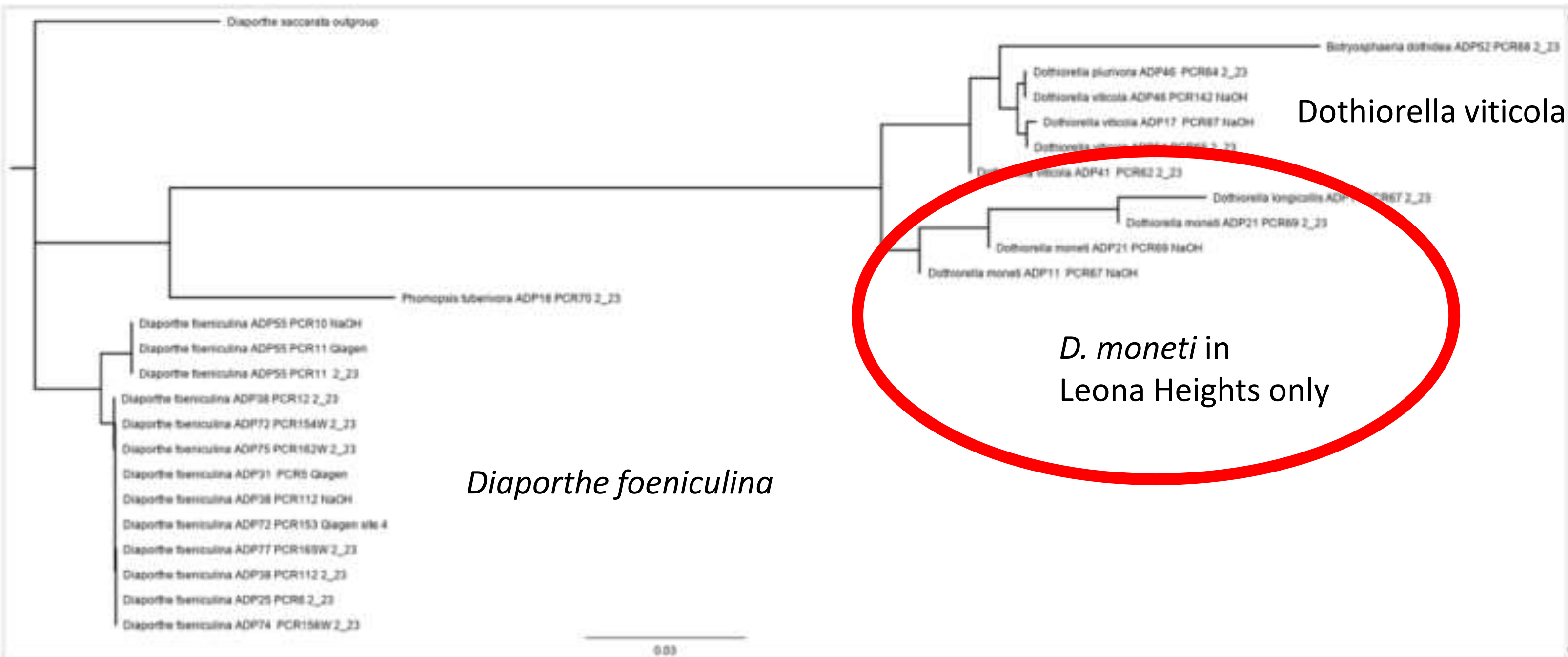


Table 2. Various information about two *Dothiorella* and *Diaporthe* species isolated in 2020/2021 from declining and dying acacias in the SF Bay Area.

Species	Reported in California	California hosts	Where else reported	Hosts outside California	Confidence in Species ID
Dothiorella moneti/ santali	No	Na	Australia	*Acacia rostrellifera, Santalum*	Medium
Dothiorella viticola	Yes	*Vitis vinicola, Citrus sinensis*	South Africa, Australia, China, Tunisia	*Vitis, Podocarpus, Prunus, Juglans, Citrus, Vachellia*	High
*Diaporthe foeniculina** **maybe includes two very closely related species*	Yes	*Citrus latifolia, Citrus limon, Salix sp., Vitis vinifera*	Southern Europe, Germany, Serbia South Africa, Uruguay, New Zealand	*Citrus, Cupressus, Diospyrus, Foeniculum Ficus, Fuchsia, Glycine, Hemerocallis, Juglans, Lumaria, Malus, Melilotus, Microcitrus, Paraserianthes, Persea, Pyrus, Prunus, Rhus, Ribes, Rosa, Salix, Vaccinum,*	Medium/High

List goes on but cut off

Primary players

These are known endophytes that turn into aggressive pathogens and then into saprobes. Complex biology makes their study and control complex

Results 2

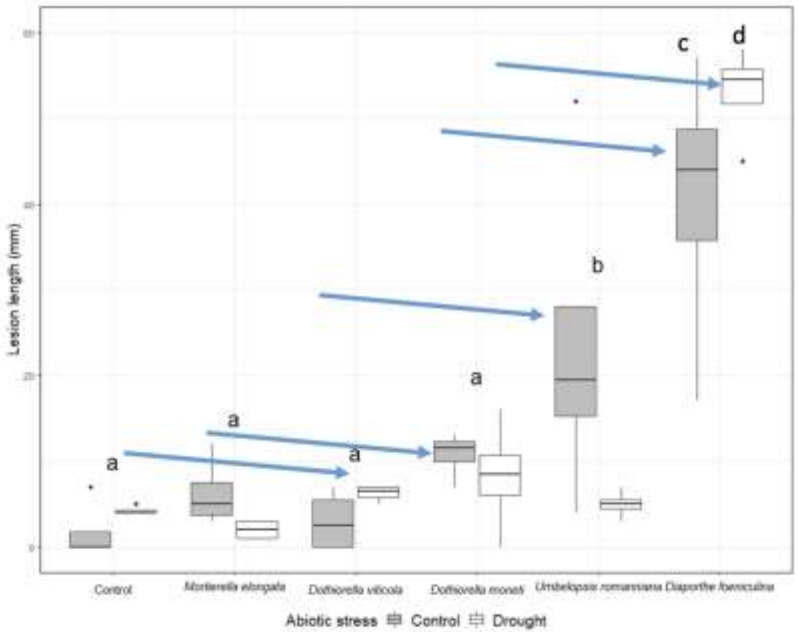
- Putative secondary fungi isolated **<u>only</u>** from trees that were also infected by *Dothiorella viticola* or *Diaporthe foeniculina*
- Only one tree was infected by both *D. viticola* and *D. foeniculina*, usually only one of the two fungi was found per tree
- Zygomycetes , known as root endophytes, normally regarded as beneficial:
  - Normally site-specific
  - However, wood symptoms were associated with their presence
  - In the SFPUC , *Umbelopsis* was found in trees **w/o** *D. viticola* or *D. foeniculina*
  - *Mortierella elongata* reported as pathogen of avocado trees

Table 3. Fungi that may further accelerate acacia decline

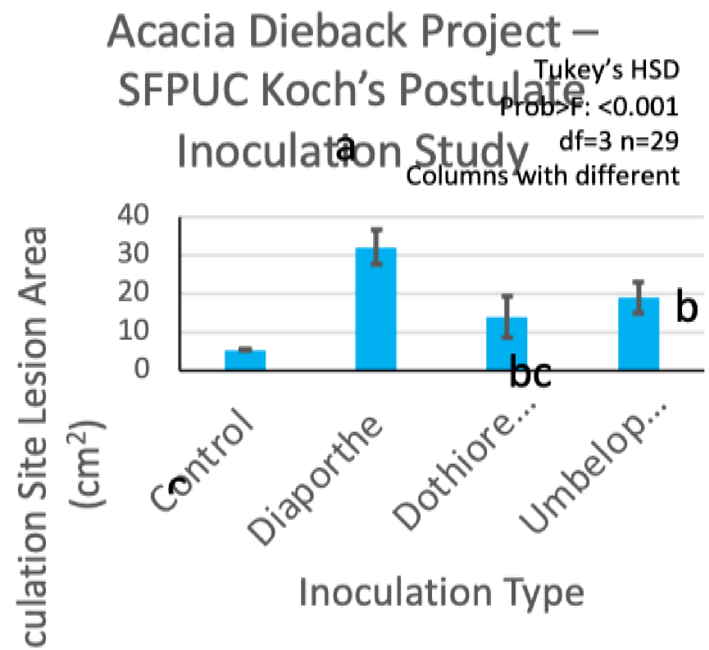
Species	Plant part affected	Symptoms	Present in California	Host(s)	Reported as pathogen	ID confidence
Fusarium oxysporum	Rootlets	n/a	Yes	Many	Yes	Low
Fusarium solani	Stem	Canker	Yes	Many	Yes	Low
Fusarium sarcochrum	Stem	Canker	No	Many	Yes	Low
Mortierella elongata	Roots/ Soil	Roots?	Yes?	Many	Once on Avocado	High
Mortierella hialina	Roots	Endophyte	?	Many	Beneficial	High
Umbelopsis ramanniana	Roots and root collar	Staining	Yes?	Tanoak, conifers	? Xylem colonization	High

SYMPTOMS OBSERVED

Acacia inoculation results



Seedlings



Trees

Other pathogens in Eucalyptus

- ***Cytospora*** spp. were isolated from three sites, typical symptoms
- The Bot fungus ***Neofusicoccum eucalyptorum***: *In 3 sites,* in twigs and branches displaying typical xylem discoloration (cankers). Broad host range (Myrtaceae and Ericaceae) May be one of the reasons why we need to reduce inoculum by disposing of debris
- A xylareaceous fungus in the genus ***Graphostroma/Biscogniauxia*** was isolated in three sites both in SFPUC and East Bay).
- The development of disease caused by all fungi above has long been known to be associated with environmental stresses and defoliation (6). All have an endophytic stage

Nine First Reports!

- ***Diaporthe foeniculina*, first report** for *A. melanoxylon*
- ***Dothiorella viticola*, first report** for *A. melanoxylon*
- ***Dothiorella moneti,* first report** for California
- ***Umbelopsis ramanniana,* first report** for *A. melanoxylon*
- ***Pseudosydowia eucalypti,* first report** in California
- ***Cytospora eucalypticola*, first report** from *E. globolus* in California
- ***Neofusicoccum eucalyptorum,* first report** for California
- ***Neofusicoccum australe,* first report** for *U. californica*
- ***N. luteum,* first report** for *U. californica*

Different types of pathogens

- **Primary pathogen**: aggressive, capable of causing disease in healthy trees, e.g. *Phytophthora ramorum*
- **Secondary or opportunistic pathogen**: capable of infecting trees whose health is severely compromised by a primary pathogen or by extreme climatic stress
- **Latent pathogen**: an endophytic microbe able to exist within plant tissue within causing symptoms for extended time periods and triggered to be an aggressive primary pathogen by changed plant physiology.

Putting the pieces of the story together: ACACIAS

- Acacias are not native to California
- *D. viticola (Dv)* and *D. foeniculina (Df)* are generalist fungi present (native?) in California. They are known endophytes that can turn pathogenic and known to cause aggressive disease
- Dv and Df performed a host jump onto acacias in the past decades
- Infection and endophytic plant colonization by both fungi known to be facilitated by high rainfall: record rainfall in 2017 led to massive infection
- High density of acacias stands facilitated spread events (no social distancing- fungi are infectious like Covid-19)
- In 2020 Dv and Df triggered to become pathogens; disease can be rapid because fungi are already inside plant! This would explain sudden onset of mortality!
- The weather itself does not explain the mortality: infectious fungi are involved

Putting the pieces of the story together: EUCs

- Eucalyptus are not native to California
- None of the fungi isolated are known as primary aggressive pathogens
- Pseudosydowia eucalypti and Neofusicoccums eucalyptorum are exotic but likely to be widespread in planted eucalyptus thanks to their endophytic stage. They are specific to Eucalyptus
- This is mostly a dieback outbreak caused by host specific fungi driven by climate

Latent pathogens

- Dr. Jekyll- Mr. Hyde
- Reversal from Hyde to Jekyll may not be possible
- Because these pathogens are already present in the host, disease can be rapid and impossible to curtail
- Climate change by itself may not be enough to kill host, so these are key players
- Previously underestimated

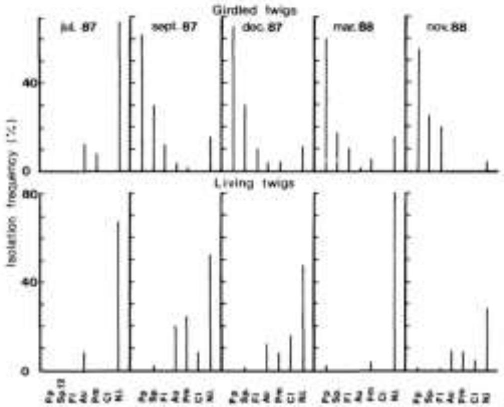


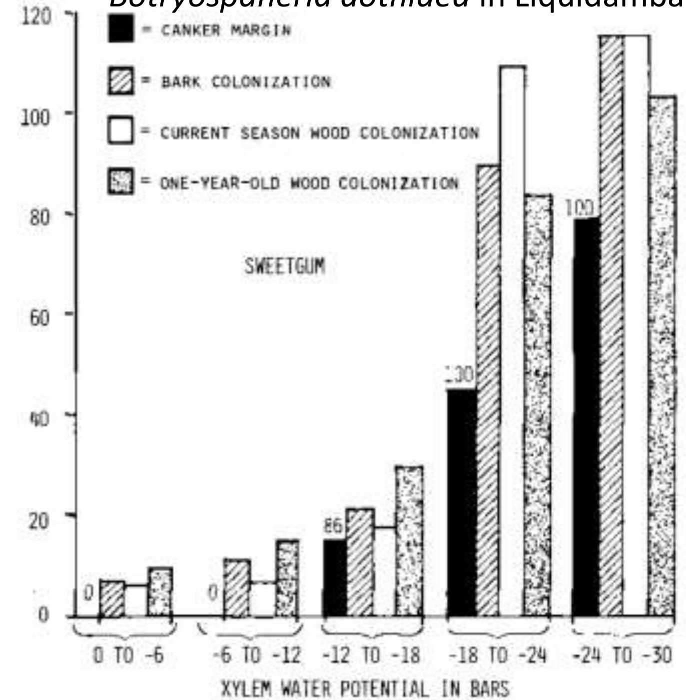
Fig 7. Isolation frequency of primary colonizers and superficial colonizers from stressed and living ash (*Fraxinus excelsior*) twigs. Abbreviations: Pm: *Phoma macrostoma* Mont.; P p: *Phomopsis platanoidis* Died.; Fl: *Fusarium lateritium* Nees; Aa: *Aureobasidium* spp.; Cl: *Cladosporium* spp.; N.I.: Nothing isolated. Data for living twigs from 25 samples per time and for girdled twigs from 75. From Griffith (1989).

Living

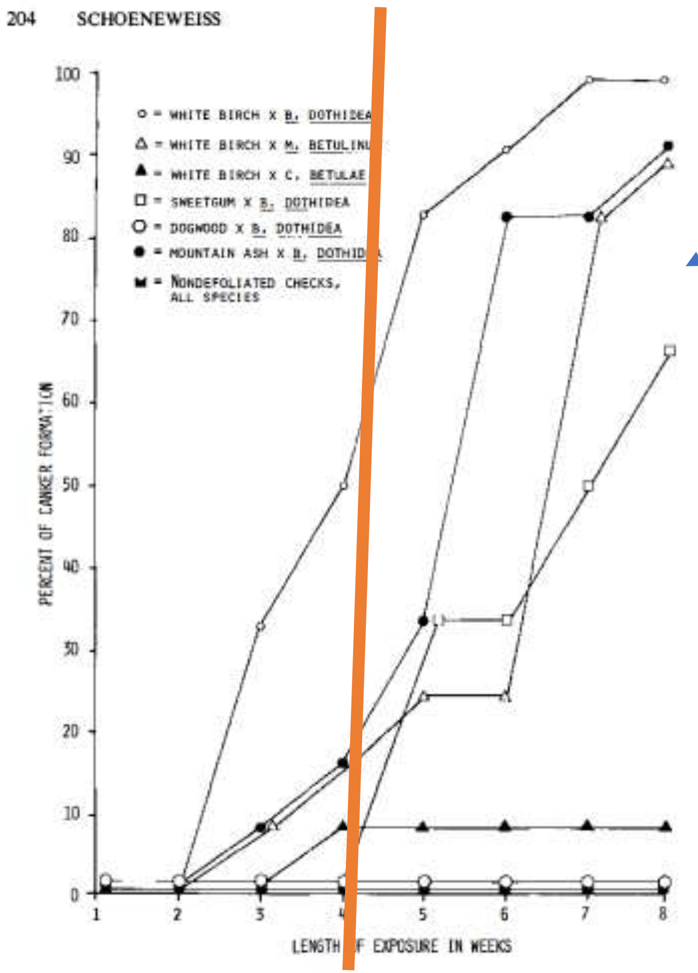
Girdled

A different fungal communities emerges
In the same twigs, when girdled (no outside input)

Botryospaheria dothidea in Liquidambar



Water potential has long been identified as the major trigger of pathogenic growth of endophytes. Endophytes require drier wood. Ascomycetes can survive In very dry wood, not so basidiomycetes, that is why Ascomycetes will succeed as saprobes on dead wood.

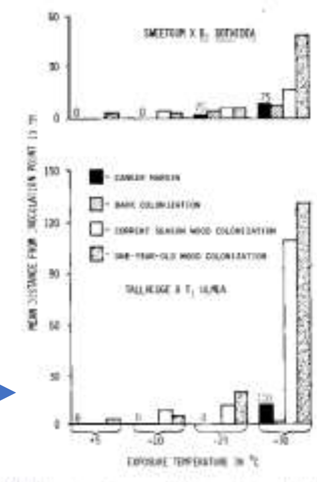


Defoliation is also a known trigger of pathogenic growth of endophytic fungi, although some tree species are more susceptible to defoliation than others

There is a threshold : in this experiment (data not shown) Re-leafing after four weeks stopped the growth of the endophytes, but not so after 5 weeks.

There is a threshold after which there is no turning back

Freezing damage can facilitate endophytic fungal growth



Is large-scale mortality sudden with latent pathogens?

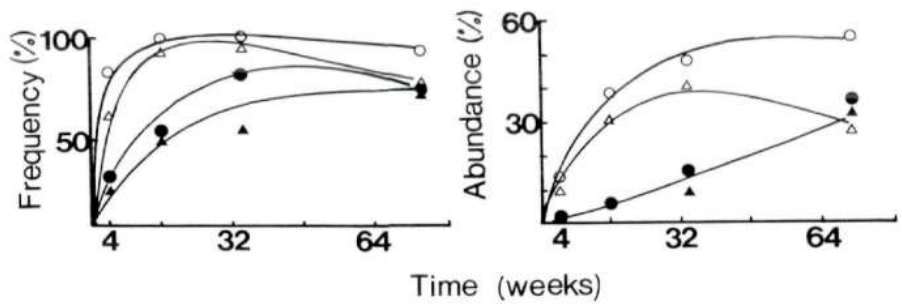


Fig. 8. Temporal changes in frequency and abundance of isolation of latently established colonizers (open symbols) and basidiomycetes (not latent in healthy branches; closed symbols) in freshly felled branches on the forest floor at two different sites (●, site 1; ▲, site 2) in S. W. Britain. From CHAPELA & BODDY (1988c). Reproduced by permission of FEMS Microbiology Ecology.

- Yes, they are, because latent pathogens are already in their host!

What will determine which endophytic pathogen will be present?

Host specificity: host and pathogen must be compatible at the physical and molecular levels. Some pathogens are strictly host specific.

Plant community composition: endophytes are infectious so if there is a source, and the pathogen in the source is not strictly host specific then you can have a host jump. Of course, **intraspecific** host jumps happen all the time.

Temperature can be a significant factor too, endophytes have very different temperature optima

How aggressive can latent pathogens be?

- It depends on conditions in the wood environment: water potential and temperature being major drivers

- Has the point of no return being crossed?

- It depends on other fungi present: turf wars and priority effects are strong in wood fungal communities

- It depends on the species of latent pathogen and on the host: some pathogens are more aggressive than others and some hosts are more susceptible than others

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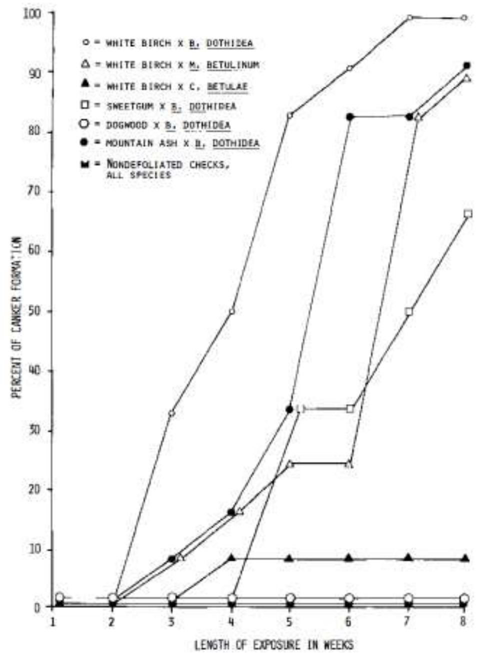


Same ENDOPHYTE, but different HOSTS

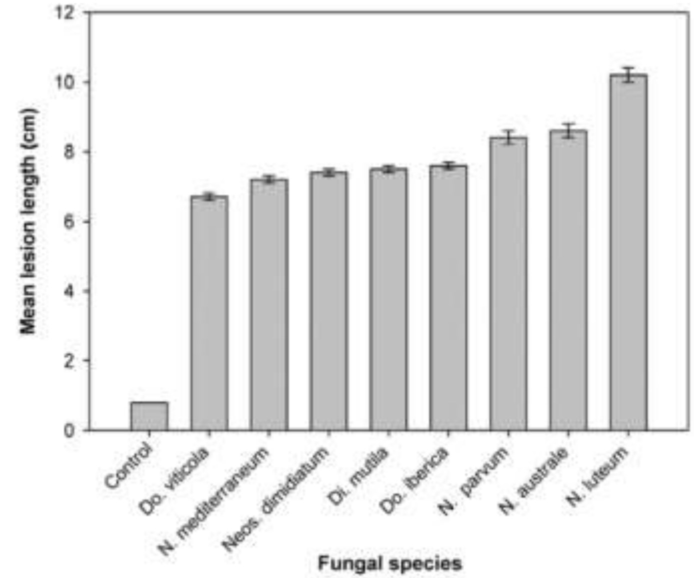


Fig. 4. Mean lesion lengths on excised 'Eureka' lemon shoots inoculated with isolates belonging to eight species of Botryosphaeriaceae. Vertical lines represent standard error of the mean according to Tukey's honestly significant difference mean separation test at $\alpha = 0.05$.

Same HOST, but different ENDOPHYTES

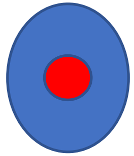
How aggressive can latent pathogens be?

- Starts from the bark or phloem

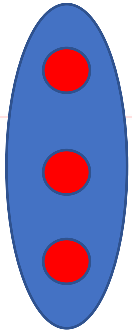
and moves into the sapwood

- It starts and stays in the sapwood

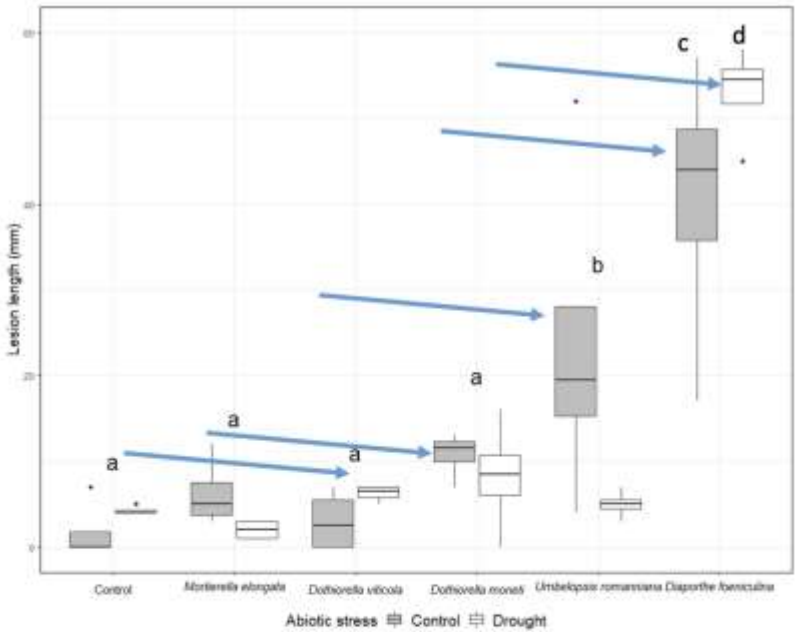
- Lesion has a unique starting point



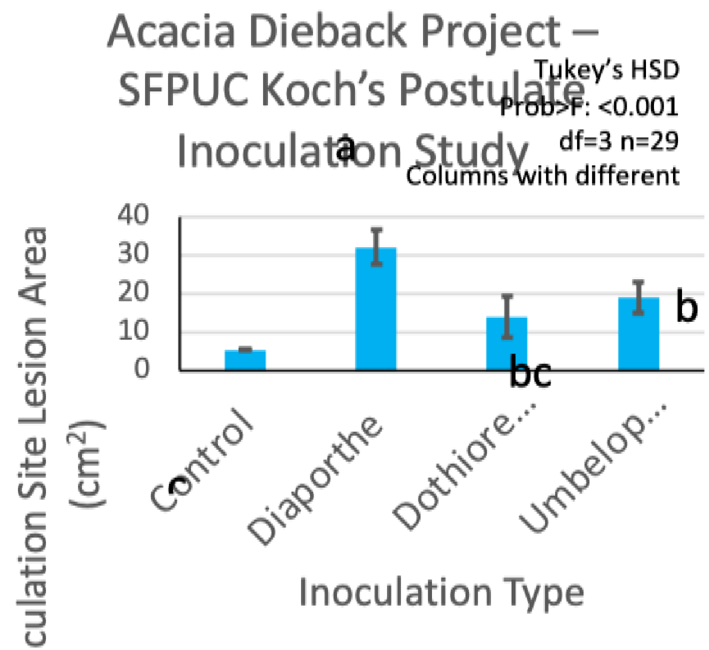
- Multiple starting points that coalesce



Acacia inculation results

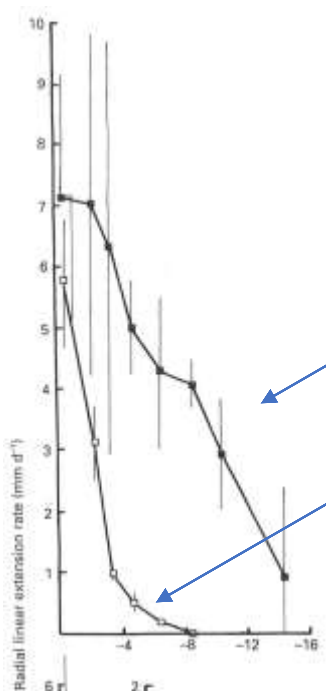


Seedlings



Trees

Ascomycetes have greater water potential tolerance than basidiomycetes

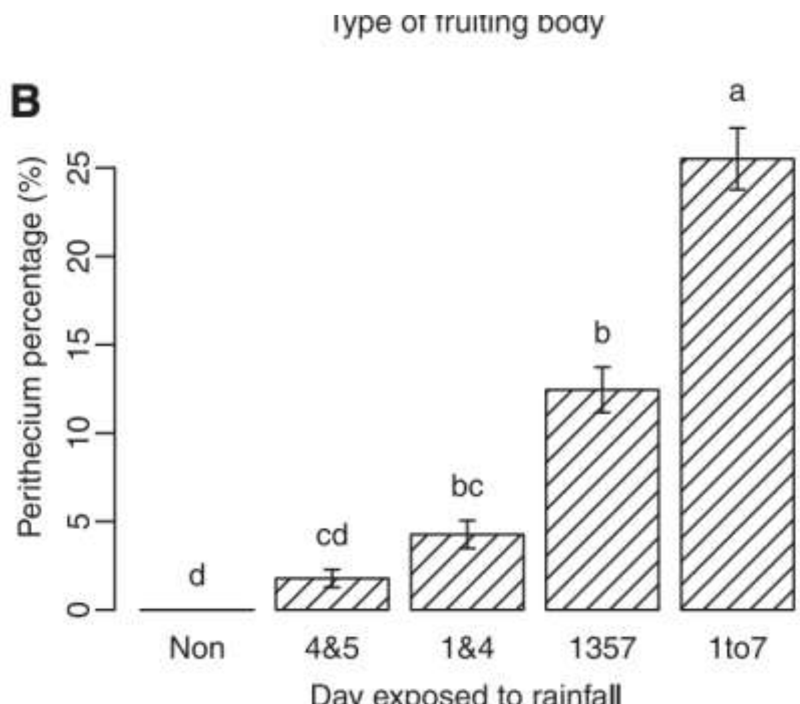


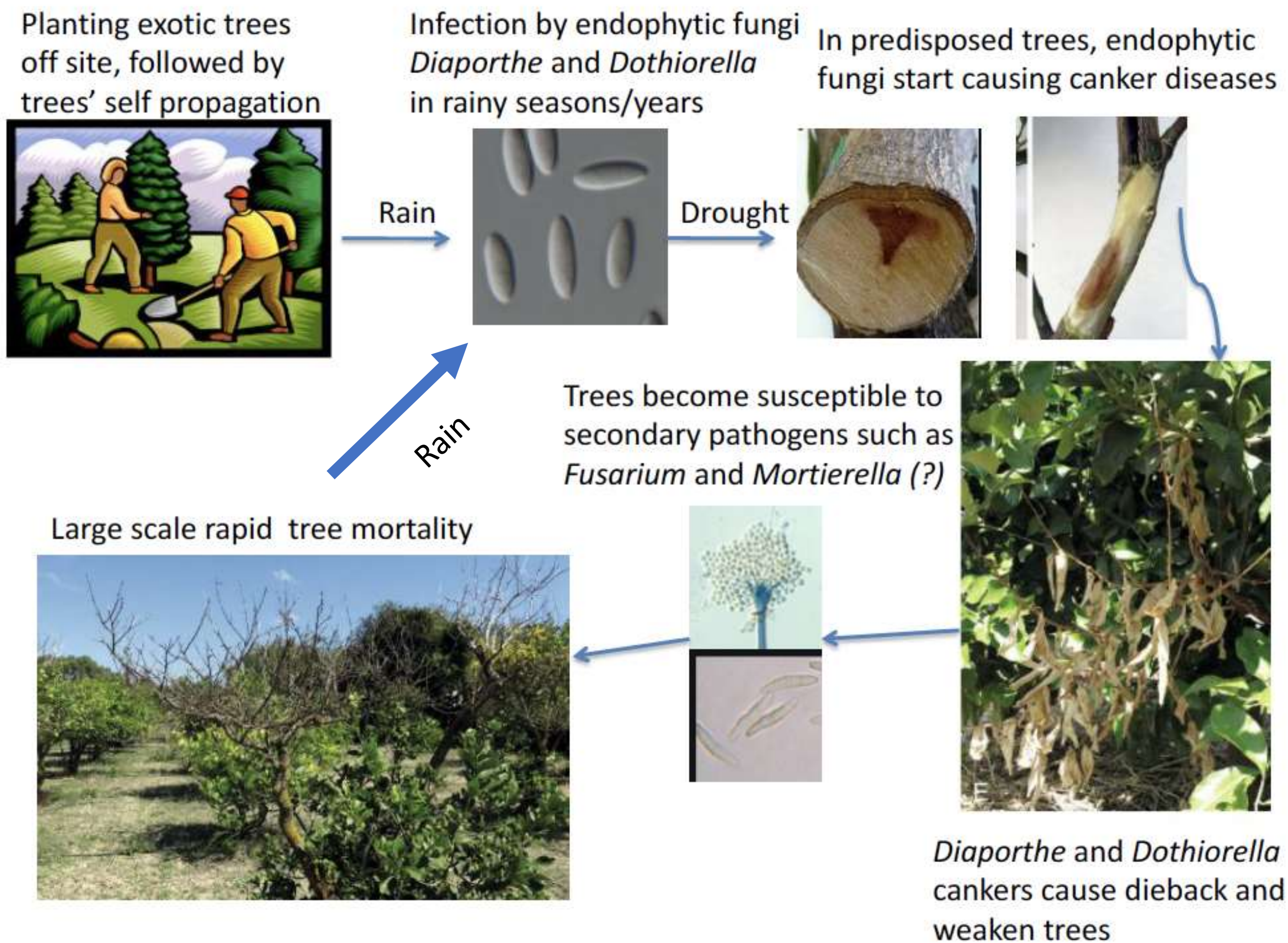
Typical ascomycete

Typical basidiomycete

Ascomycetes can survive in very dry wood, not so basidiomycetes, that is why ascomycetes will succeed as saprobes on dead wood.

And the more rain, the more spores….




Planting exotic trees off site, followed by trees' self propagation
Rain
Infection by endophytic fungi *Diaporthe* and *Dothiorella* in rainy seasons/years
Drought
In predisposed trees, endophytic fungi start causing canker diseases
Diaporthe and *Dothiorella* cankers cause dieback and weaken trees
Trees become susceptible to secondary pathogens such as *Fusarium* and *Mortierella (?)*
Large scale rapid tree mortality
Rain

DISEASE AS THE RESULT OF CLIMATE CHANGE AS A DISTURBANCE: ARE THE CONSEQUENCES PERMANENT (RED LINE) , TEMPORARY (DOTTED LINE) OR CYCLICAL WITH A TREND (YELLOW LINE)?

Forests 2017, *8*, 147

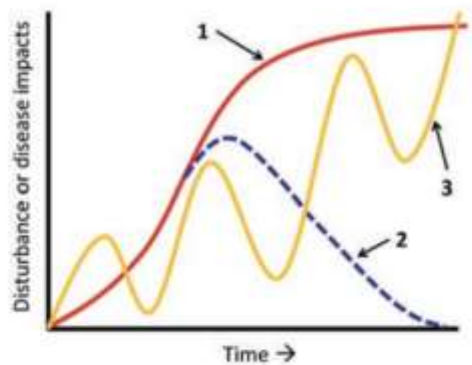


Figure 2. Several examples of disturbance and disease interactive outcomes over time. Impacts may increase and reach a new stable state (1—red); increases may gradually recover to pre-outbreak levels (2—blue dashed); or feedbacks between disease and disturbance could create fluctuating dynamics over time, here depicted as an oscillating, but increasing impact (3—yellow).

Review

Tree Diseases as a Cause and Consequence of Interacting Forest Disturbances

THE TIMES

The war on nature

Published at 12:00AM, April 15 2004

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Trees become casualties of war

SCIENCE

THE WAR ON NATURE

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THE CLUES CAME FROM THE ESTATE'S ARCHIVES

CAN THE STONE PINE SURVIVE THE ONSLAUGHT?