



# 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

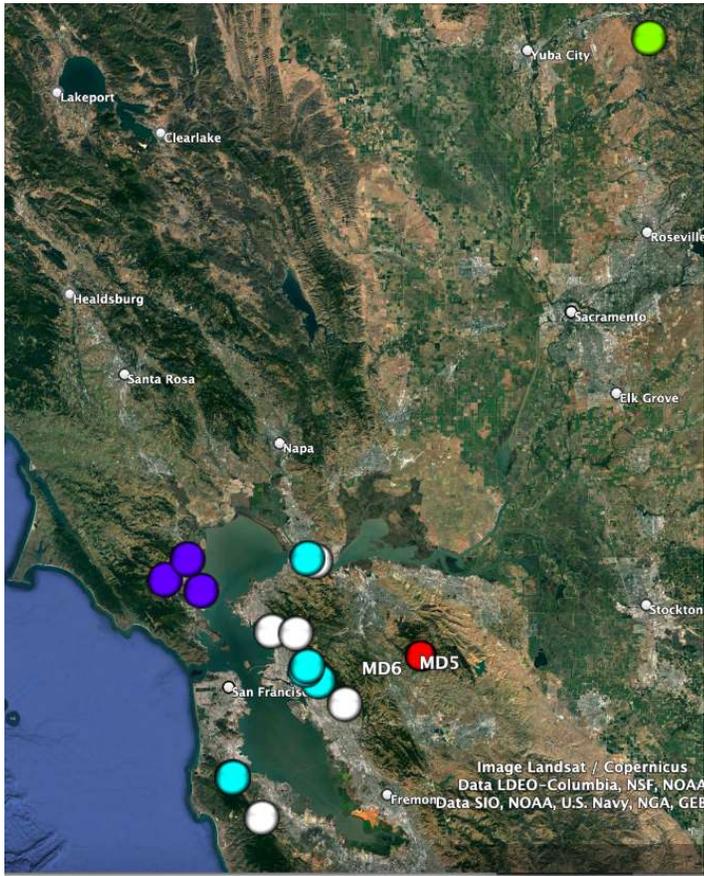
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## Objectives: Understanding the drivers of the large-scale dieback of exotic and native species observed in Northern California

- 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



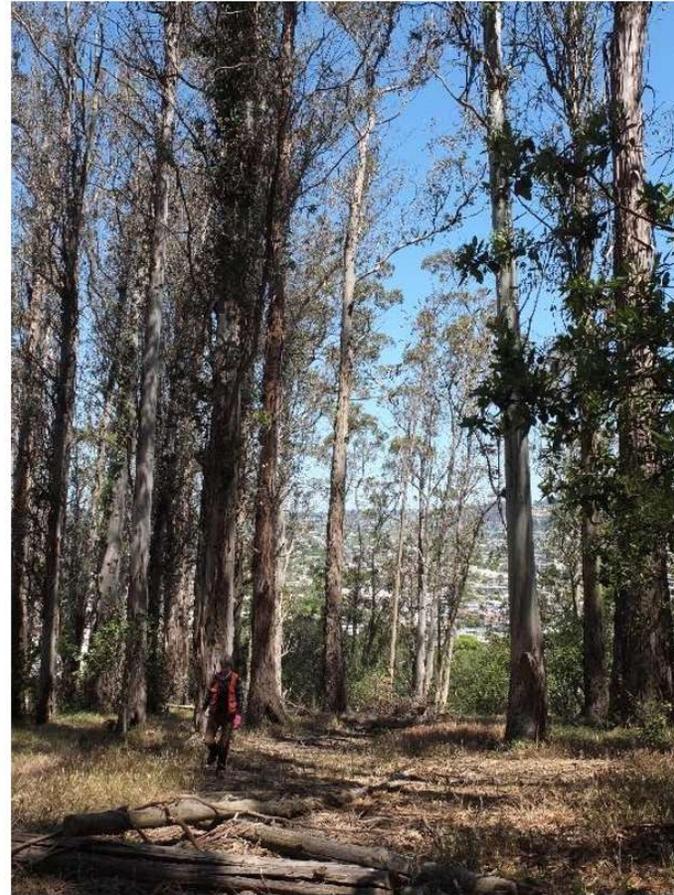
Leona Heights, Oakland, April 2021. Acacia dieback

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

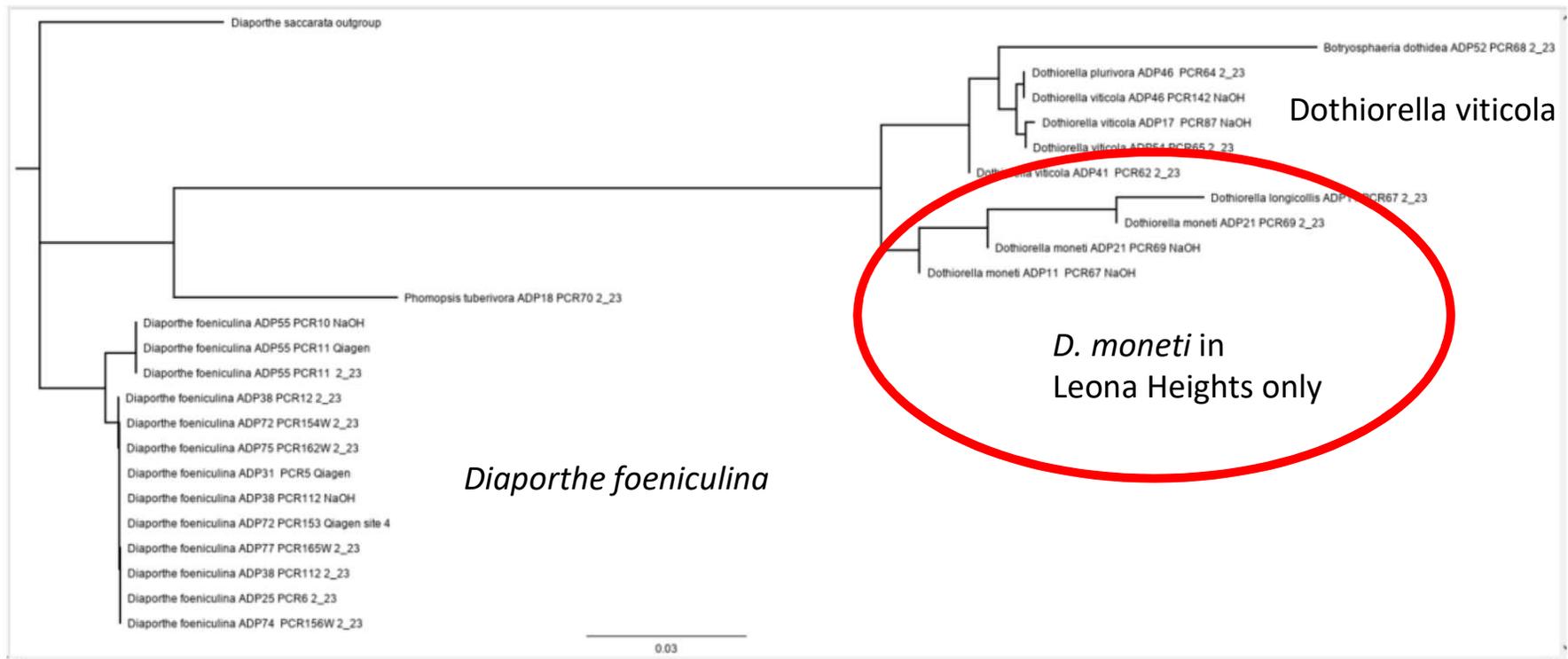
- ***Dothiorella moneti***: a pathogen of acacias, exclusively reported from Australia. Known latent pathogen with endophytic stage (inside plant but not causing disease), likely to be moved where acacias are moved
- Greenhouse inoculations showed it to be slightly more aggressive than *D. viticola*,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

- Eucalyptus

- ***Pseudosydowia eucalypti***: although unreported in Ca it is an endophyte and a fungus of unknown virulence, unofficially known to be ubiquitous where Eucalyptus grows
- ***Neofusicoccum eucalyptorum***: unreported in California, but found only 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
<i>Dothiorella moneti/santali</i>	No	Na	Australia	<i>Acacia rostellifera</i> , <i>Santalum</i>	Medium
<i>Dothiorella viticola</i>	Yes	<i>Vitis vinicola</i> , <i>Citrus sinensis</i>	South Africa, Australia, China, Tunisia	<i>Vitis</i> , <i>Podocarpus</i> , <i>Prunus</i> , <i>Juglans</i> , <i>Citrus</i> , <i>Vachellia</i>	High
<i>Diaporthe foeniculina</i> *  <small>*maybe includes two very closely related species</small>	Yes	<i>Citrus latifolia</i> , <i>Citrus limon</i> , <i>Salix sp.</i> , <i>Vitis vinifera</i>	Southern Europe, Germany, Serbia South Africa, Uruguay, New Zealand	<i>Citrus</i> , <i>Cupressus</i> , <i>Diospyrus</i> , <i>Foeniculum</i> <i>Ficus</i> , <i>Fuchsia</i> , <i>Glycine</i> , <i>Hemerocallis</i> , <i>Juglans</i> , <i>Lumaria</i> , <i>Malus</i> , <i>Melilotus</i> , <i>Microcitrus</i> , <i>Paraserianthes</i> , <i>Persea</i> , <i>Pyrus</i> , <i>Prunus</i> , <i>Rhus</i> , <i>Ribes</i> , <i>Rosa</i> , <i>Salix</i> , <i>Vaccinum</i> ,	Medium/High

## Primary players

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

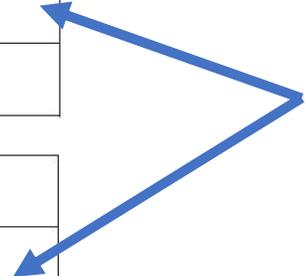
List goes on but cut off

## Results 2

- Putative secondary fungi isolated **only** 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

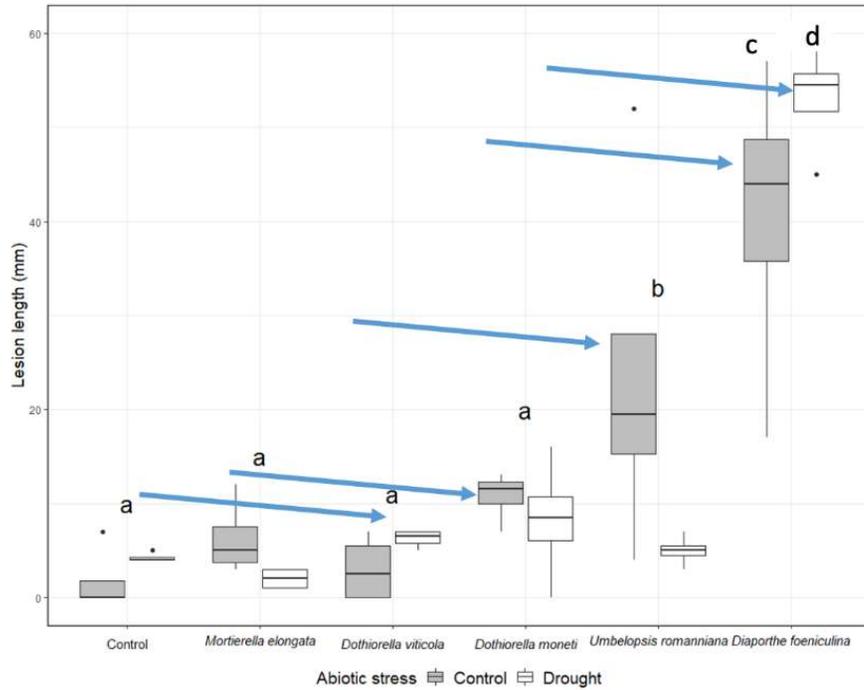
**Table 3.** Fungi that may further accelerate acacia decline

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

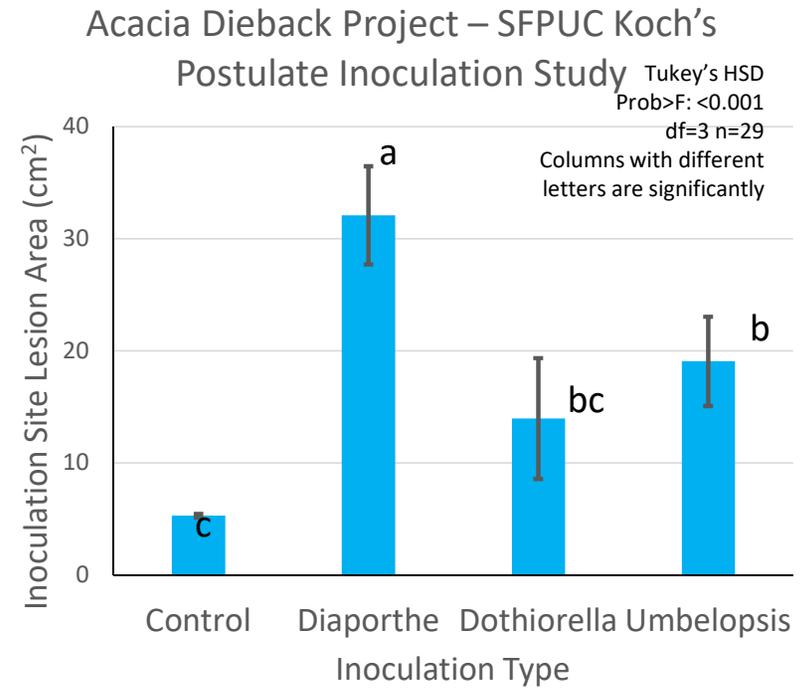


SYMPTOMS OBSERVED

## 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. melanoxydon***
- ***Dothiorella viticola*, first report for *A. melanoxydon***
- ***Dothiorella moneti*, first report for California**
- ***Umbelopsis ramanniana*, first report for *A. melanoxydon***
- ***Pseudosydowia eucalypti*, first report in California**
- ***Cytospora eucalypticola*, first report from *E. globulus* 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 without 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

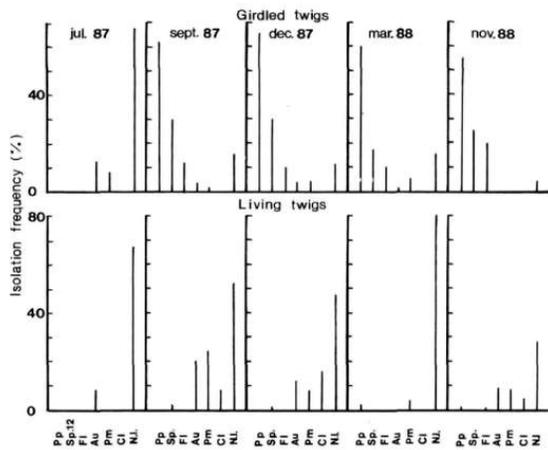
# Putting the pieces of the story together: EUCs

- Eucalyptus are not native to California
- None of the fungi isolated are known as primary aggressive pathogens
- *Pseudosydowia eucalypti* and *Neofusicoccum 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



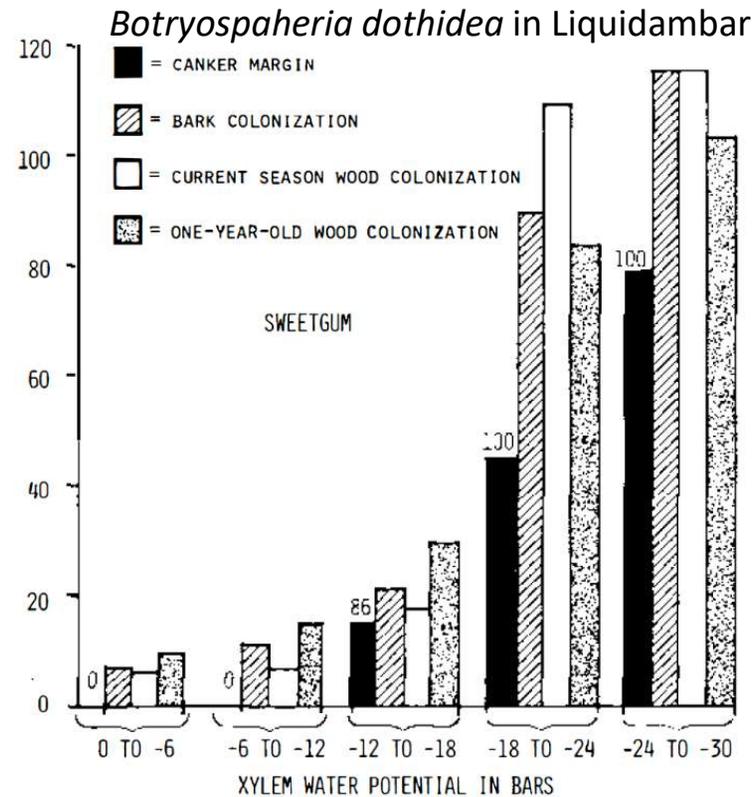


Living

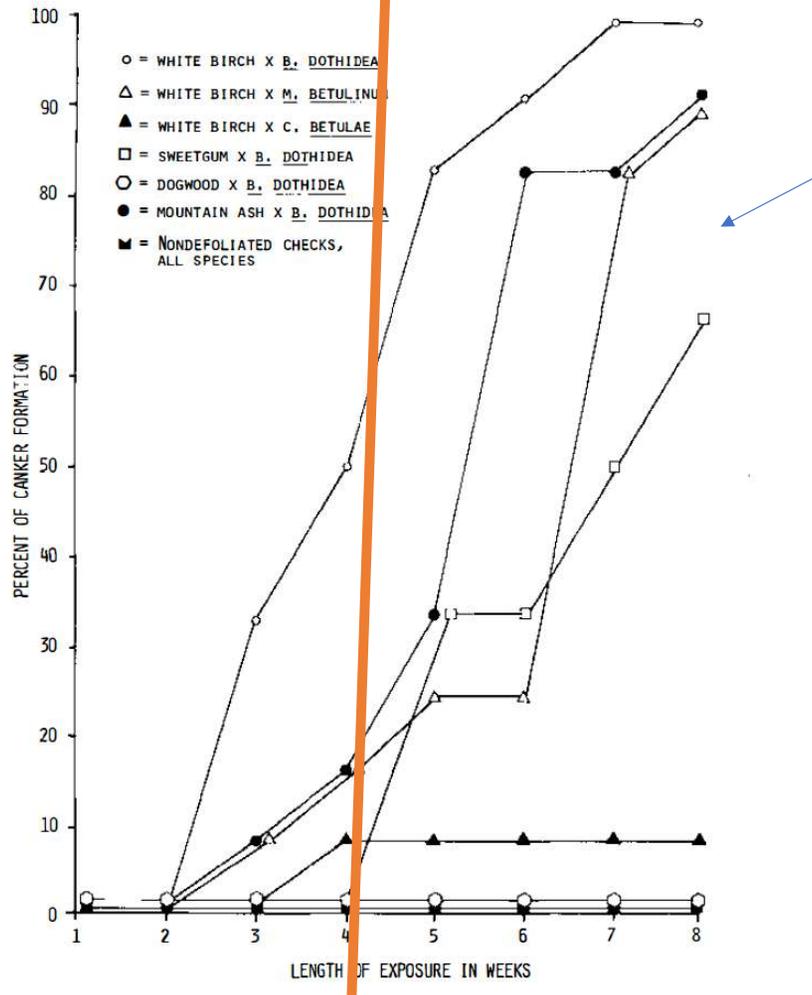
Girdled

Fig. 7. Isolation frequency of primary colonizers and superficial colonizers from stressed and living ash (*Fraxinus excelsior*) twigs. Abbreviations: Pm: *Phoma macrostoma* MONT.; Pp: *Phomopsis platanoideis* DIED.; F1: *Fusarium lateritium* NESS.; Au: *Aureobasidium* spp.; C1: *Cladosporium* spp.; N.L.: Nothing isolated. Data for living twigs from 25 samples per time and for girdled twigs from 75. From GRIFFITH (1989).

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



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.

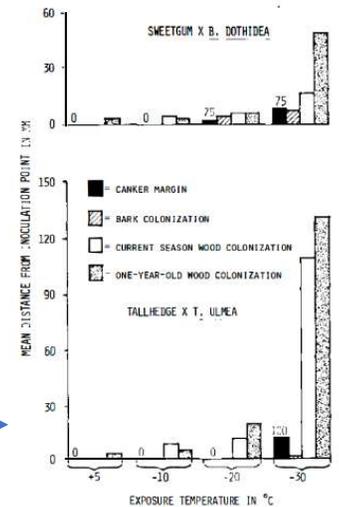


Defoliation is also a known trigger of pathogenic growth of endophytic fungi, although some tree species are more susceptible to defoliation than others

There is a threshold : in this experiment (data not shown) Re-leaving 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?

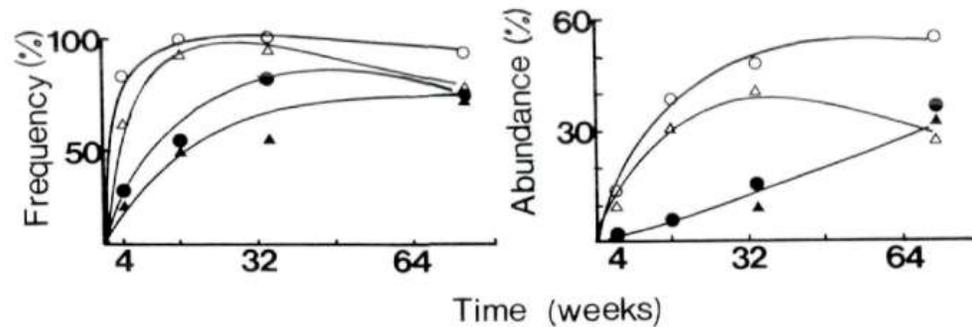


Fig. 8. Temporal changes in frequency and abundance of isolation of latently established colonizers (open symbols) and basidiomycetes (not latent in healthy branches; closed symbols) in freshly felled branches on the forest floor at two different sites (●, site 1; ▲, site 2) in S. W. Britain. From CHAPELA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

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



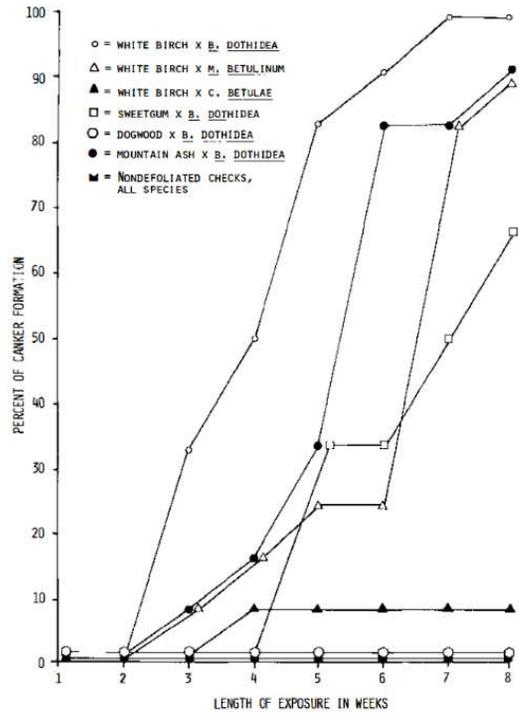
Plant community composition: endophytes are infectious so if there is a source, and the pathogen in the source is not strictly host specific then you can have a host jump. Of course, **intraspecific** host jumps happen all the time.



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



Same ENDOPHYTE, but different HOSTS

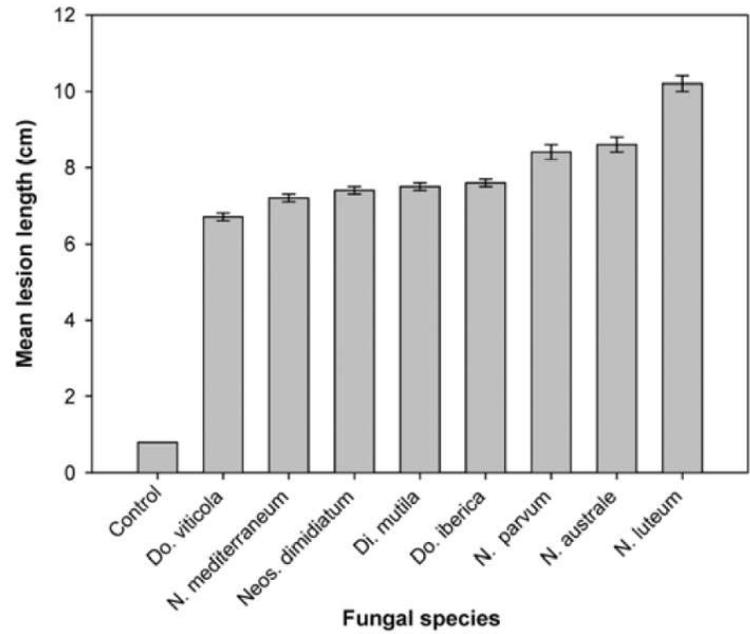


Fig. 4. Mean lesion lengths on excised 'Eureka' lemon shoots inoculated with isolates belonging to eight species of Botryosphaeriaceae. Vertical lines represent standard error of the mean according to Tukey's honestly significant difference mean separation test at  $\alpha = 0.05$ .

Same HOST, but different ENDOPHYTES

# How aggressive can latent pathogens be?

- Starts from the bark or phloem and moves into the sapwood



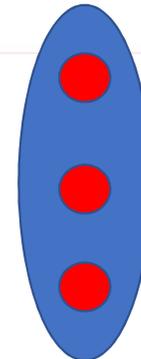
- It starts and stays in the sapwood



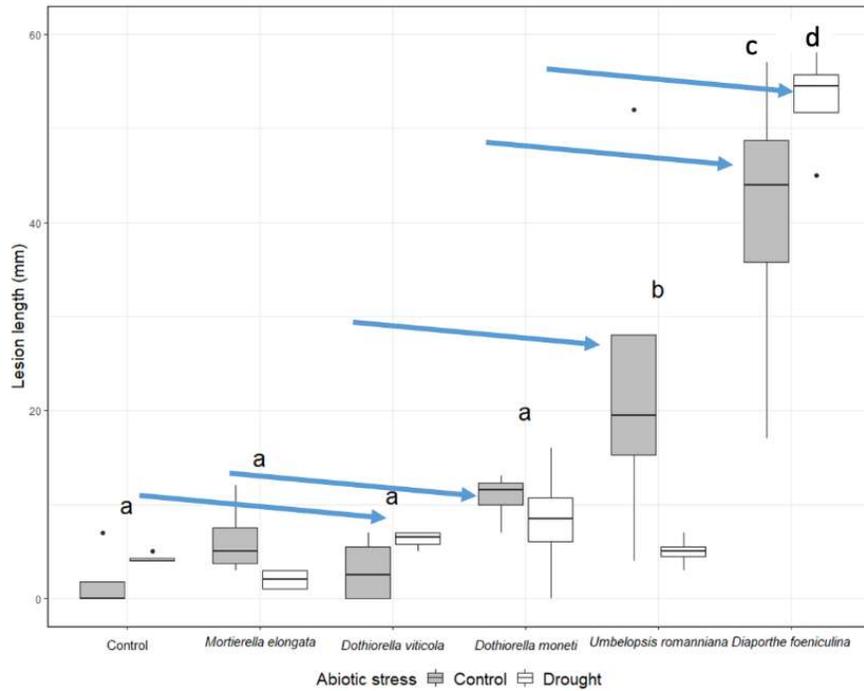
- Lesion has a unique starting point



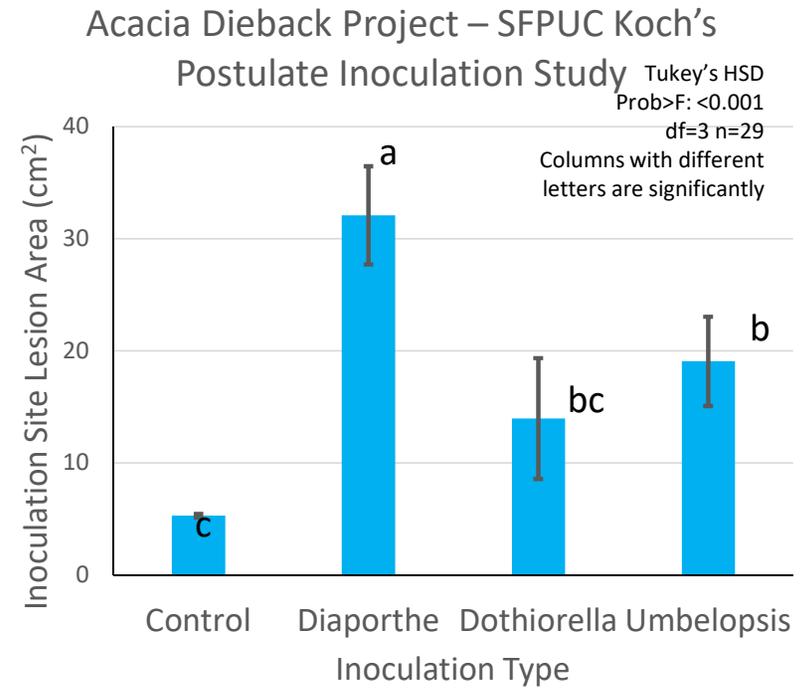
- Multiple starting points that coalesce



## Acacia inoculation results

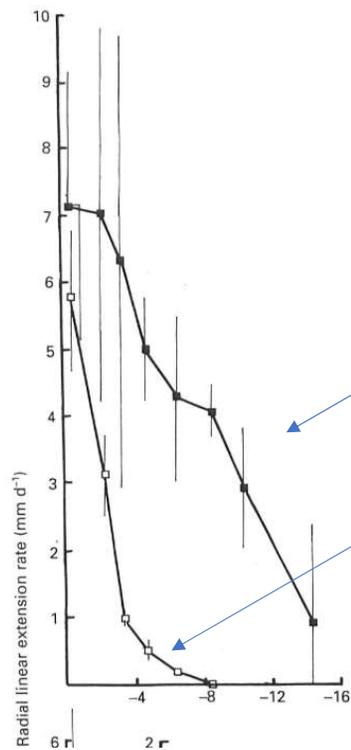


Seedlings



Trees

# Ascomycetes have greater water potential tolerance than basidiomycetes

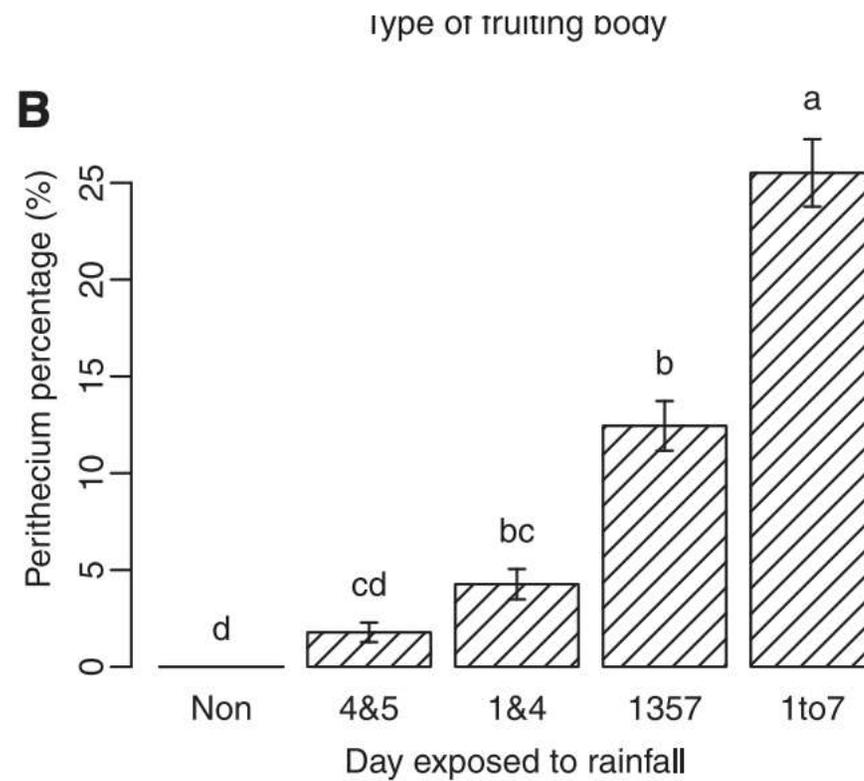


Typical ascomycete

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



Infection by endophytic fungi *Diaporthe* and *Dothiorella* in rainy seasons/years



In predisposed trees, endophytic fungi start causing canker diseases

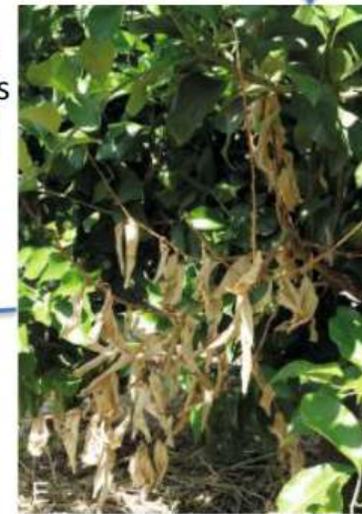


Rain

Drought

Rain

Trees become susceptible to secondary pathogens such as *Fusarium* and *Mortierella* (?)



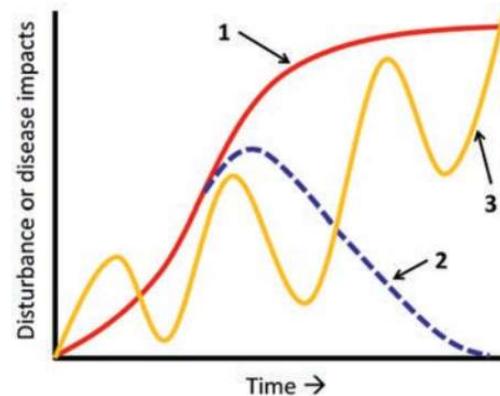
Large scale rapid tree mortality



*Diaporthe* and *Dothiorella* cankers cause dieback and weaken trees

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**

BMPs? These are not “all or nothing” recommendations and aimed at making trees happy and sanitation

- Eliminate Eucalyptus and Acacias from drier sites, where they do not belong
- In sites where these species have to be maintained (e.g. Stern Grove):
  - remove the weakest trees, identifiable by symptoms severity to reduce density
  - remove debris, burn it, compost it, mix with soil to avoid inoculum build up
  - water, making sure water does not touch canopy
  - prune trees, even severely
- For important individual specimens: prune, eliminate all green waste, water

# BMPs. Acacia vs. Eucs vs. Natives

- Fungi associated with Acacia dieback are generalists and known to cause disease (although not exotic) on a large range of plants including natives
- They sporulate on dead woody debris, so reduction of woody debris is recommended
- Mostly present in branches and stems, so focus on woody debris is important
- Although non native fungi associated with dieback they are not known to be aggressive pathogens and are Eucalyptus specific
- Although inoculum reduction is always good, in this case may not be necessary
- Most infections are in leaves and twigs, so disposal can focus on this type of green waste

# Acknowledgements

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