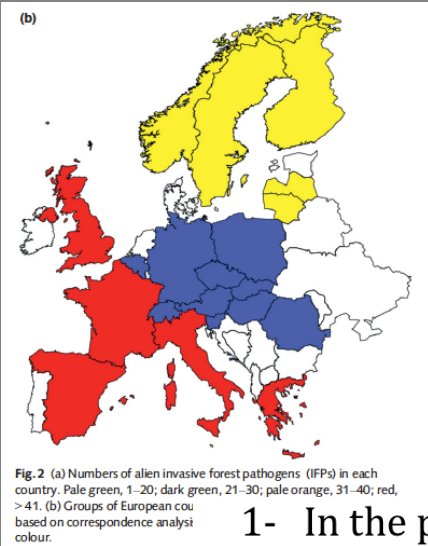
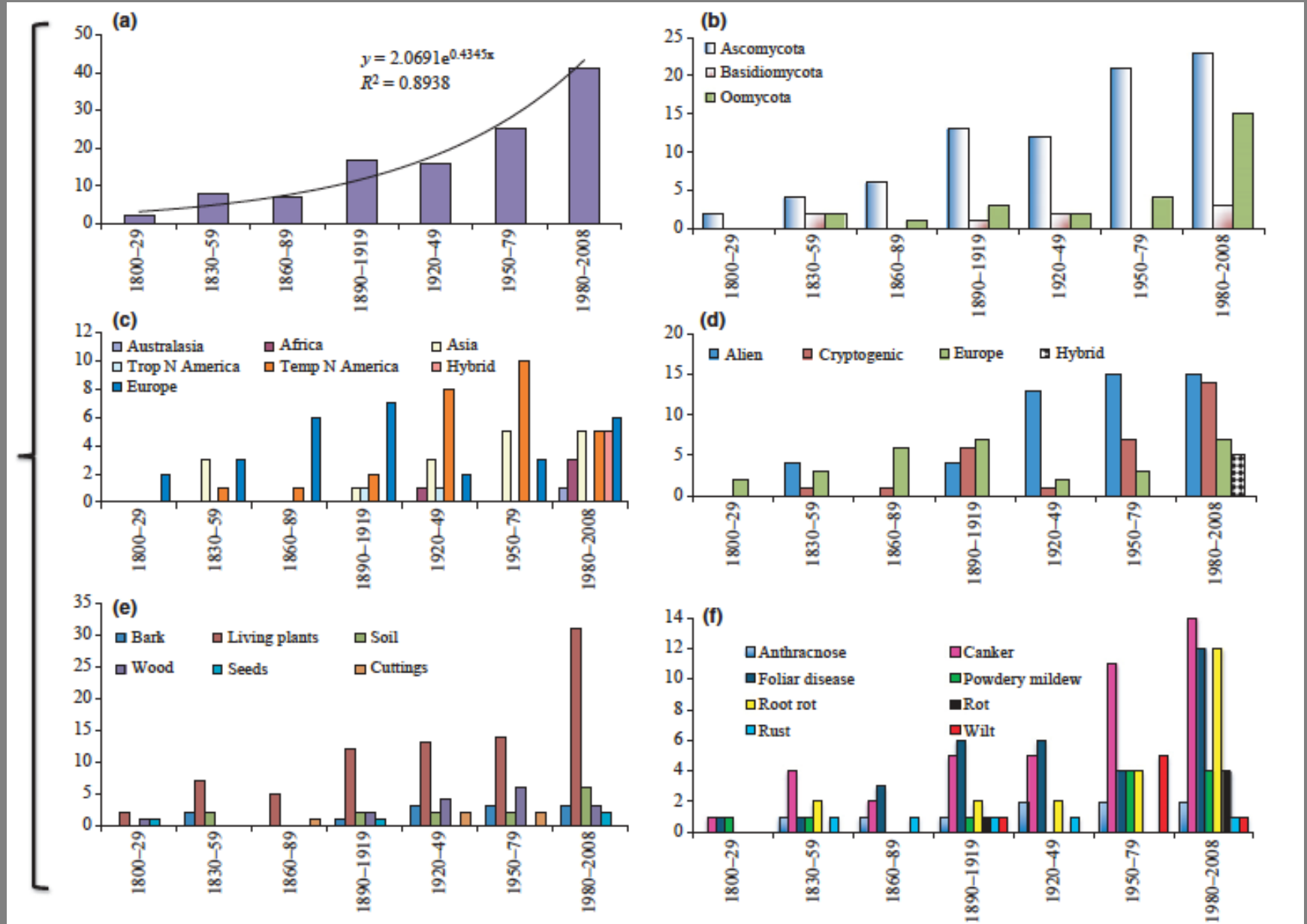


7th Quizzes



- 1- In the picture from the paper by Santini et al three different colors are highlighted: what do they mean? And why those countries are coded with different colors
- 2- Which one is the most efficient mean of introduction of pathogens in Europe according to the analysis of Santini et al
- 3- Cypress canker: why we think it is native to California?
- 4- How do we know that humans have transported *Seiridium cardinale* long distance

Descriptive stats of IFPs



“Emergent diseases”:

1: host

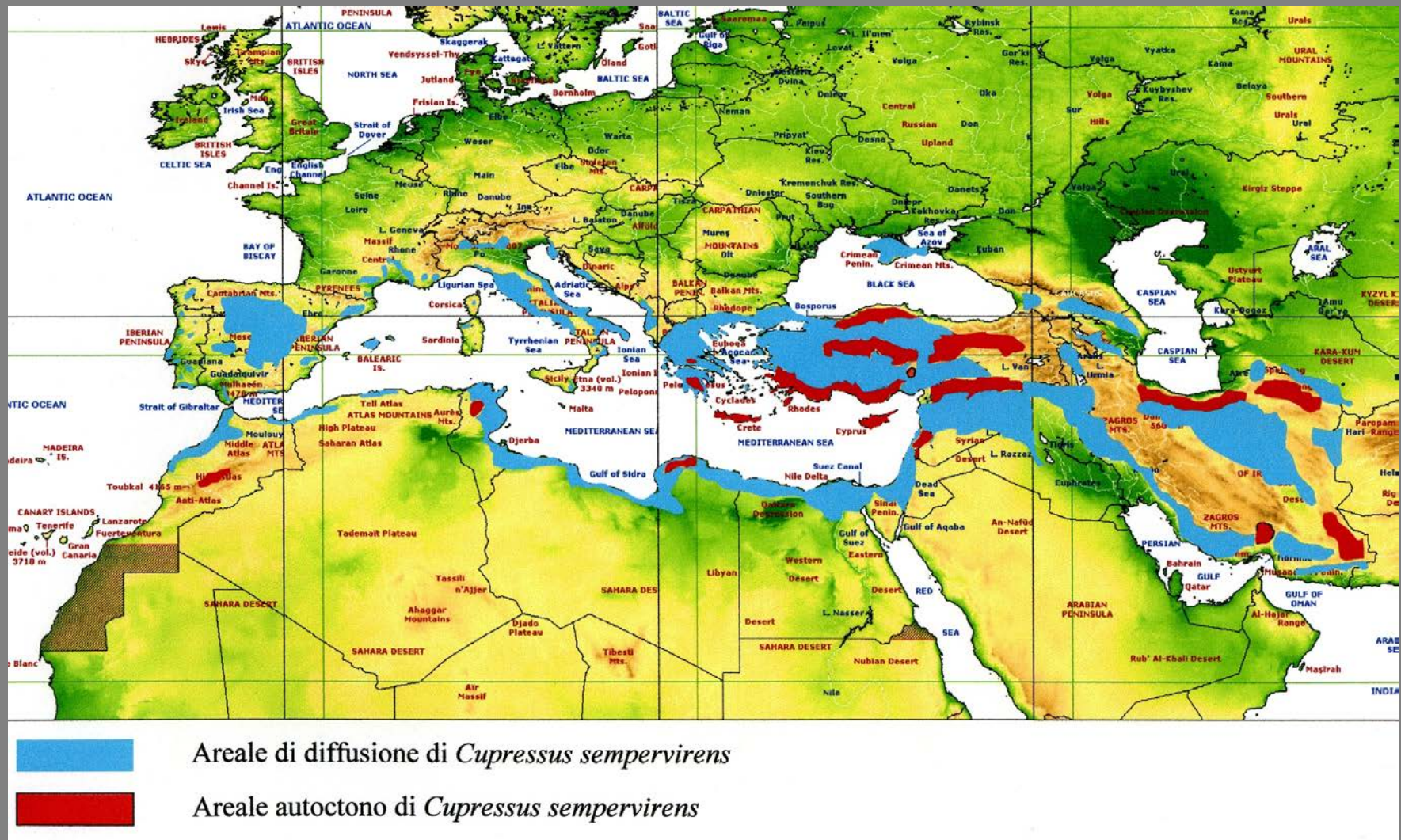
- **New host-pathogen combinations:**

exotic hosts

hosts planted off site

Cypress canker by *Seiridium cardinale*

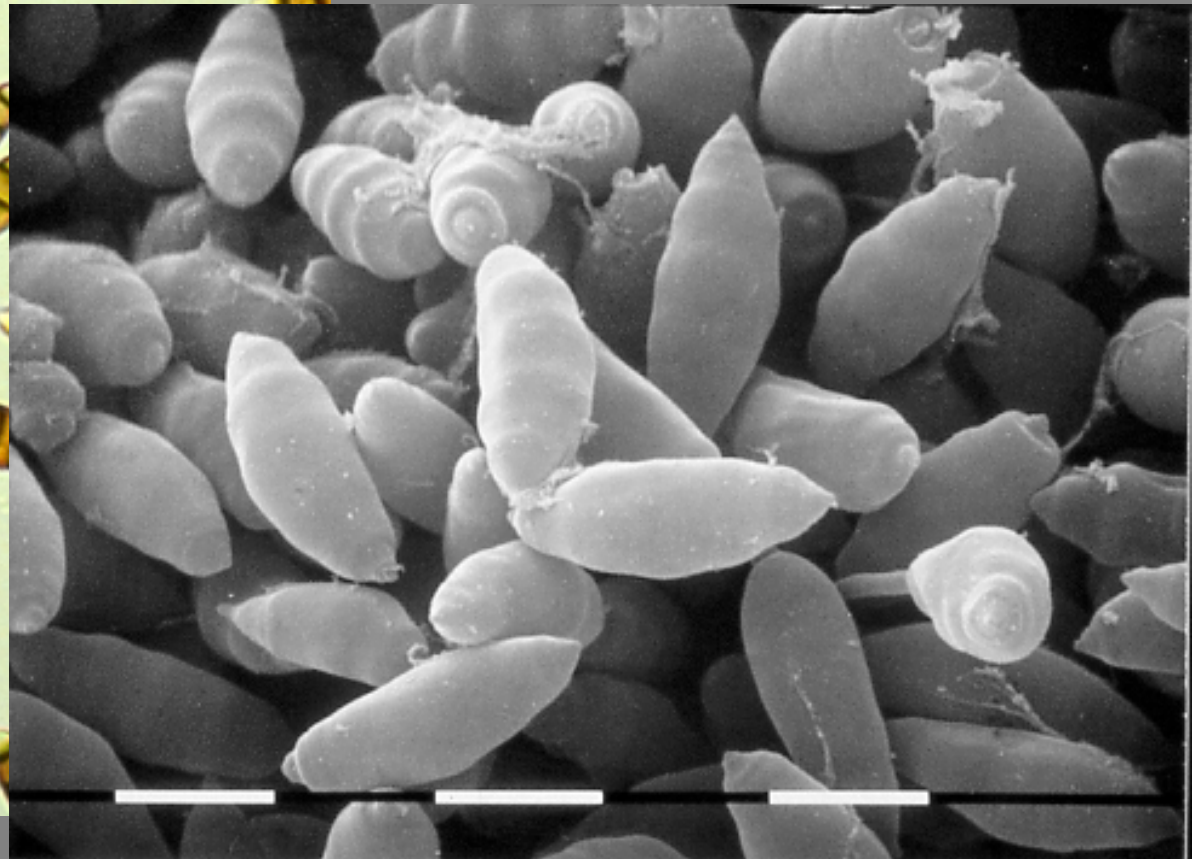
- Pathogen was first described in California in the 20s. Later it was described in Italy where it started a serious epidemic of Italian cypress
- Belief that pathogen is native to California: is that true and why is it then causing a significant disease in our state?



Natural geographic distribution of *Cupressus sempervirens* (red).

Anthropic distribution of *Cupressus sempervirens* (blue).

Conidia of *Seiridium*
cardinale observed by
optical microscope
and SEM



Needs wounds

Canker kills cambium

Toxin affects stomatal opening



**In some places of
Tuscany the situation of
Cypresses is dramatic...**

RESULTS: CA vs. Europe

- o California population diverse genetically
- o California population has a genetic structure in agreement with sexual reproduction
- o European population reproducing clonally
- o European population show less diversity



Fig. 1.



Distribution of *Seiridium cardinale* according to Wagener in 1939

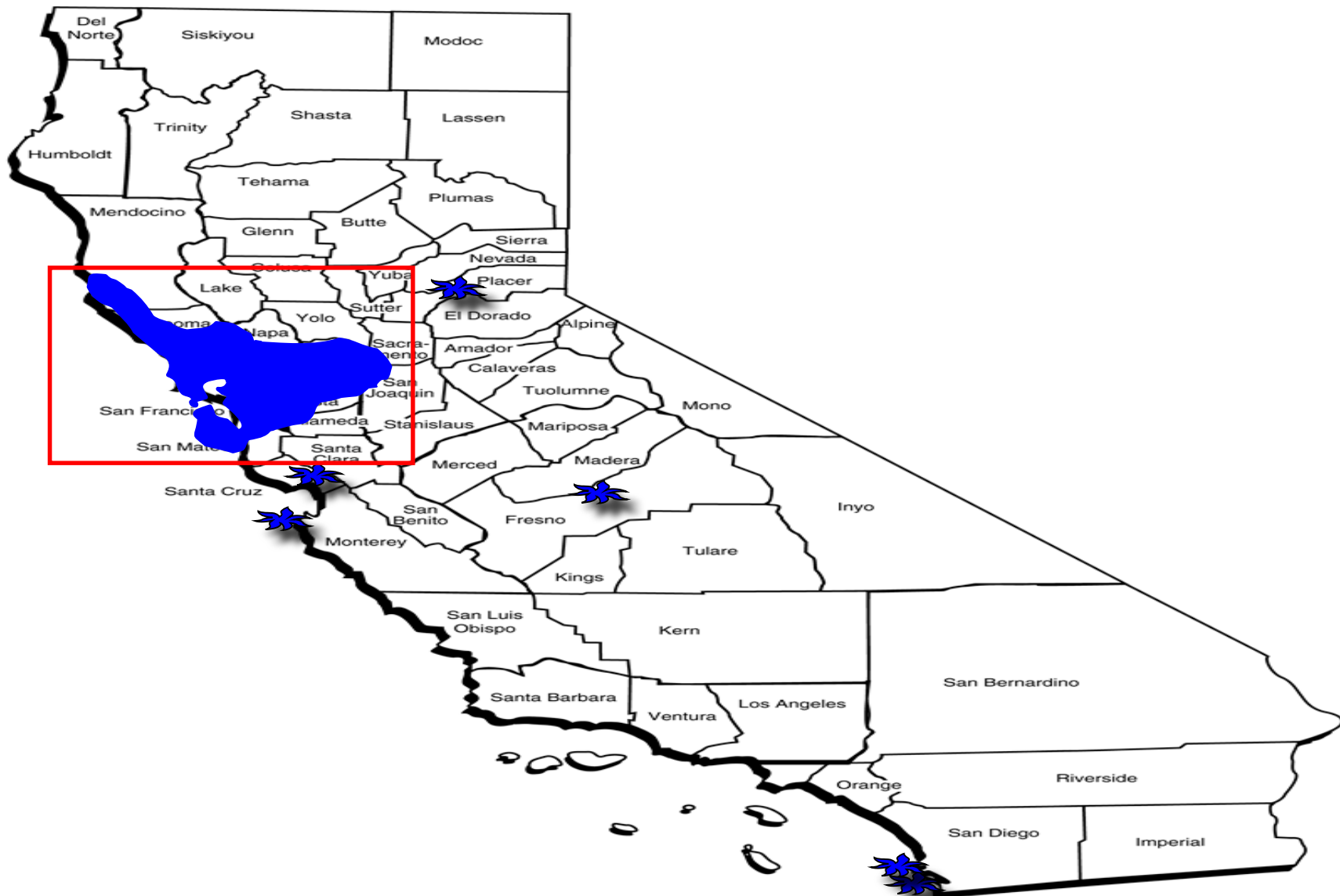


Fig. 5.

Presence of pathogen confirmed in 2008

Symptoms observed without intense surveying in 2008

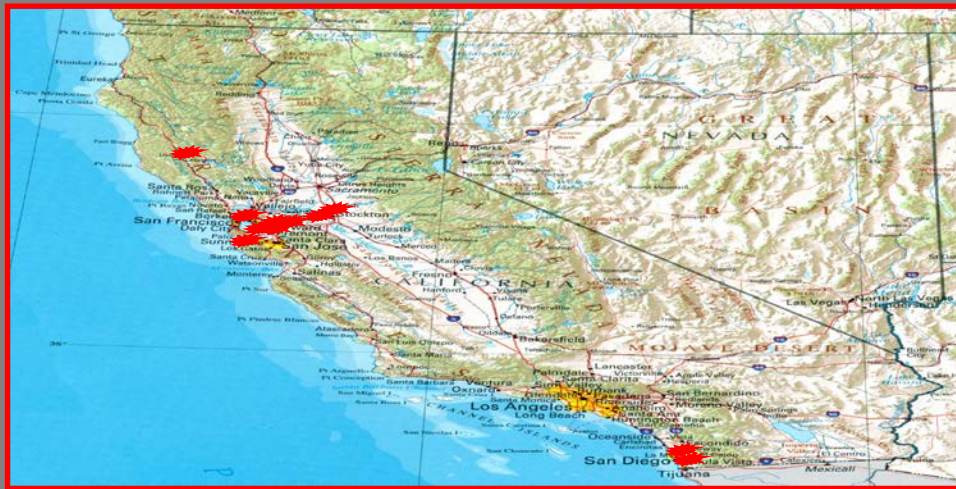


Fig. 2.
Distrinction of pathogen where intensively surveyed



Fig. 3
Symptoms caused by *Seiridium cardinale* on *Cupressus macrocarpa* (above) and *xCupressocyparis leylandii* (right)





Fig. 4.
Close-ups of symptoms leading to mortality

Why a disease in CA?

- If pathogen is native to California, why is it causing such a serious disease?
- We observed that disease incidence is variable with:
 - cypress species,
 - location,
 - abundance of planted cypresses

Range of susceptibility

- Leyland cypress, Italian, monterey are listed as most susceptible
- Arizona and McKnob are regarded as more resistant

Range of susceptibility

- 90% of Leyland are heavily infected
- 10% of monterey
- LEYLAND CYPRESS IS AN ORNAMENTAL CROSS, NOT NATIVE

Range of susceptibility

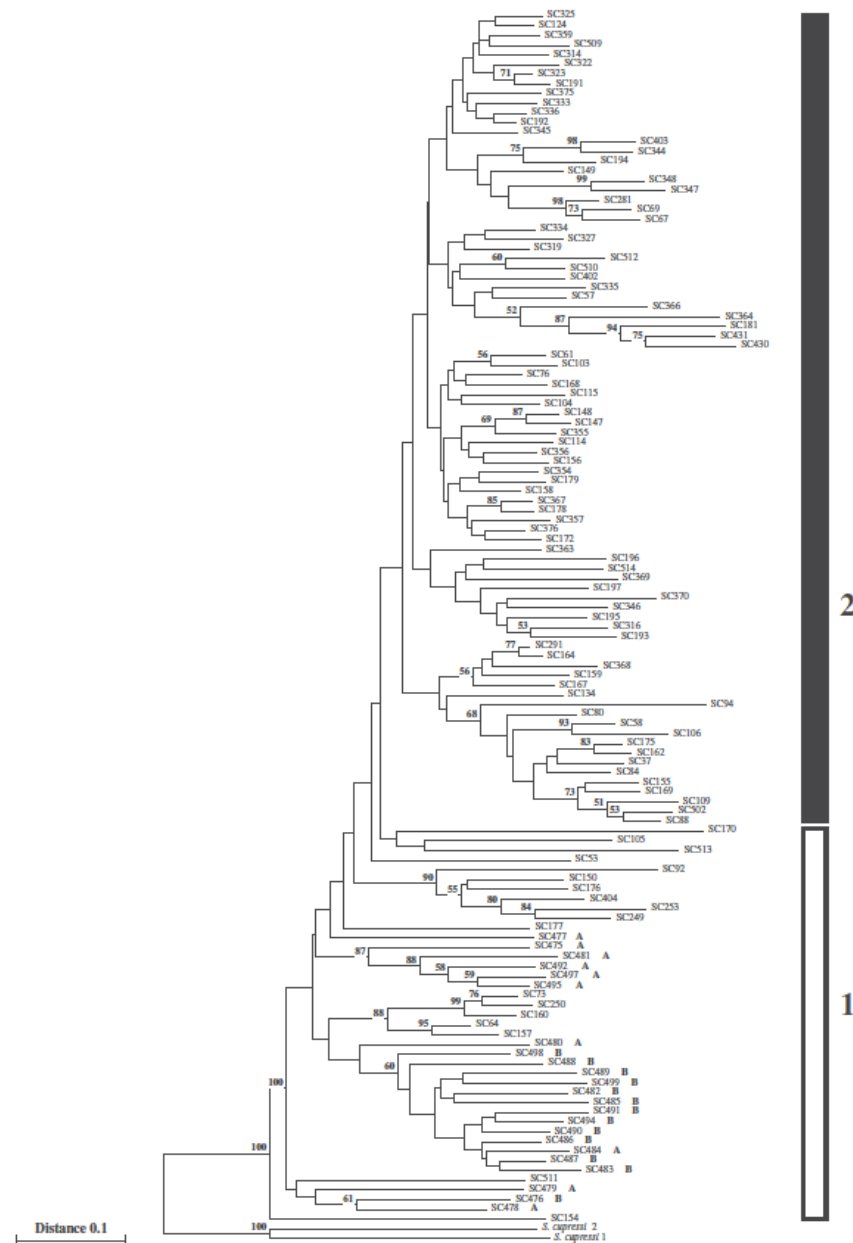
- Monterey is more susceptible in inland areas where it is NOT NATIVE: we believe that colder temperatures cause more wounds that lead to infection
- More disease where there are more cypresses: classical DENSITY DEPENDANCE effect

One consequence of an outbreak of an emergent disease...

- It may become source for an introduction

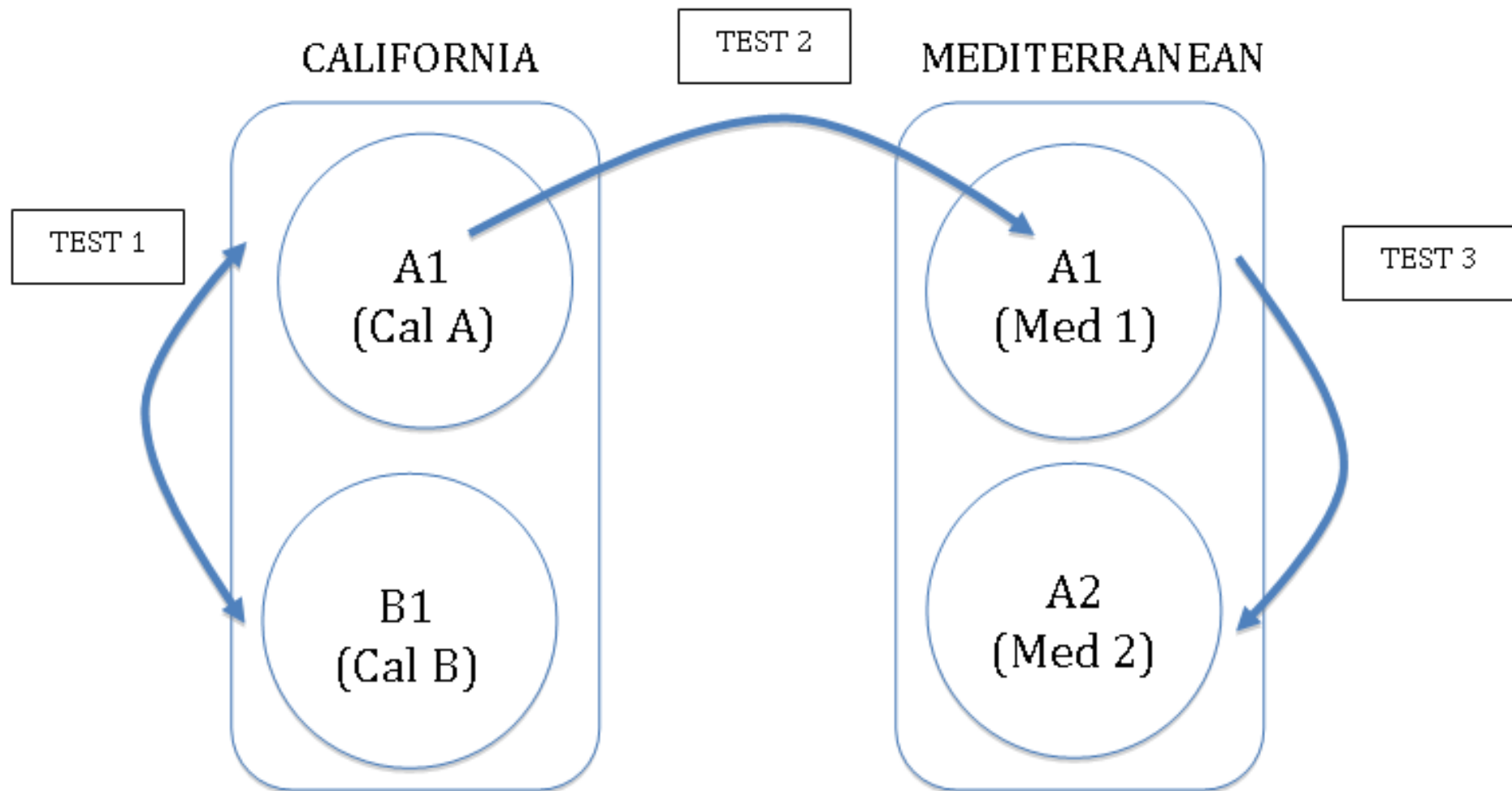
CONCLUSIONS

- Cypress canker is a serious disease in Europe because pathogen was introduced
- Cypress canker is a serious disease in California because hosts were introduced either through planting off range (Monterey cypress) or because host is artificial creation (Leyland cypress); extinction of LEYLAND is most likely



AFLP analysis identified 2 clusters in Europe, one that includes also California isolates, and one that does not, meaning it is exclusively European

Fig. 2. NJ trees calculated using pairwise Jaccard genetic similarity coefficients (S_j). Bootstrap values >50% appear above branches. Lateral bars (1 and 2) indicate clusters inferred in the structure analysis. Letters A and B indicate the β -tubulin haplotype of each Californian isolate. All Mediterranean isolates belong to the haplotype A.



We now feel comfortable stating that

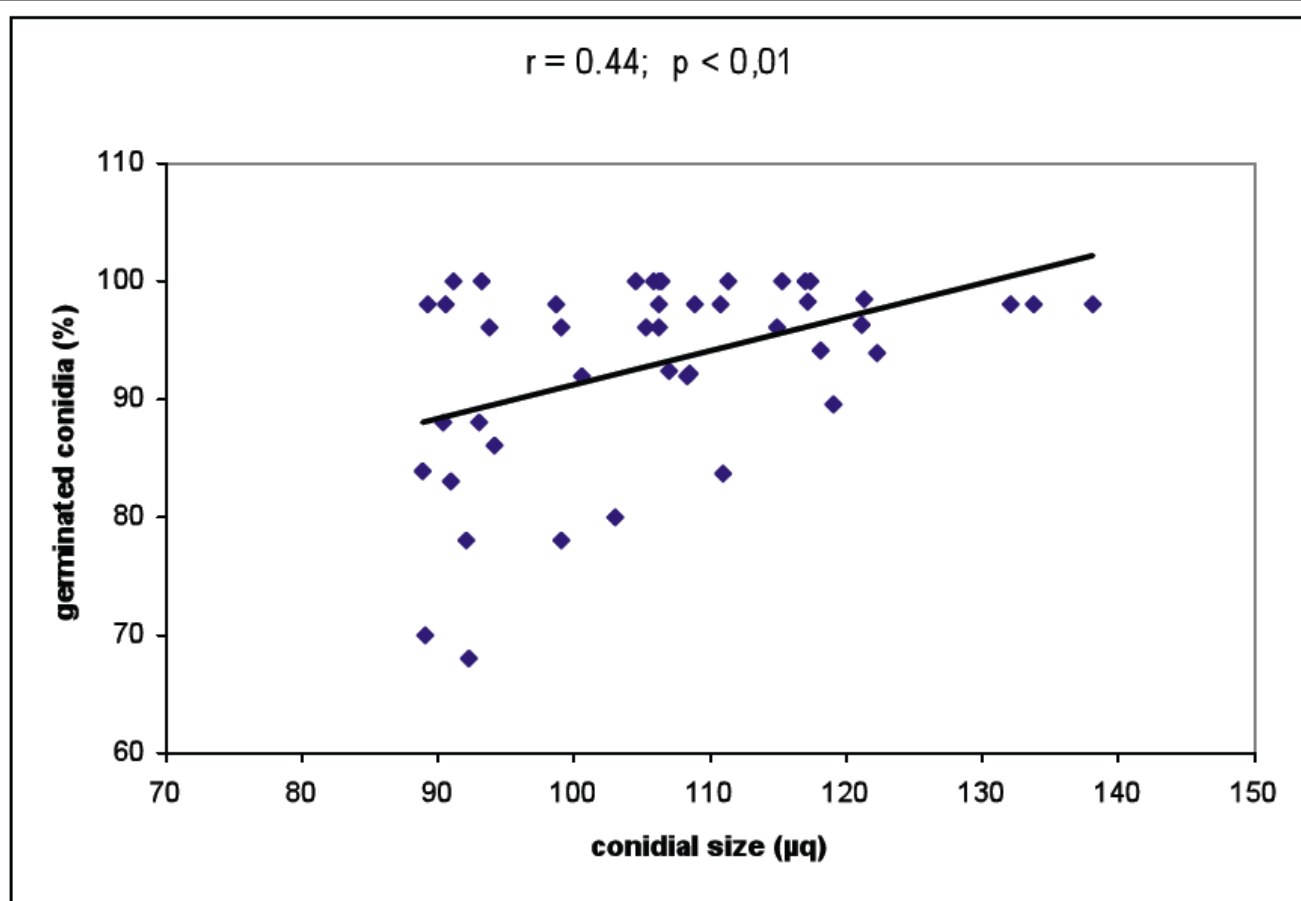
California population is the source

Mediterranean cluster 1 is the founder population

Mediterranean cluster 2 is an adaptation in the
Mediterranean

We can ask interesting questions on why *S. cardinale*
has been so successful as an invasive.

Smaller spore size is selected for and adaptive in both Med1 and Med2 in spite of trade off of reduced germination of smaller spores



Demographic plasticity increases in MED1 respect to CA, But then decreases in MED2, as the invasive adapts to Certain habitats

	Geographic population		β -tubulin Haplotype (California isolates)		Structure group (Mediterranean isolates)	
	CAL	MED	Cal A	Cal B	Med 1	Med 2
Radial growth (cm)						
15°C	0.52 (0.16)	0.66 (0.29)	0.49 (0.15)	0.22 (0.16)	0.62 (0.23)	0.64 (0.39)
20°C	0.36 (0.10)	0.49 (0.18)	0.31 (0.11)	0.29 (0.08)	0.37 (0.16)	0.45 (0.21)
25°C	0.26 (0.07)	0.48 (0.18)	0.15 (0.05)	0.26 (0.07)	0.47 (0.19)	0.39 (0.16)
30°C	0.75 (0.34)	0.70 (0.32)	0.54 (0.31)	0.74 (0.37)	0.71 (0.34)	0.62 (0.30)
Acervuli production						
30 days	1.00 (0.67)	1 (0.72)	1.00 (0.70)	0.77 (0.64)	1.00 (0.72)	0.50 (0.56)
90 days	0.81 (0.46)	0.86 (0.55)	0.79 (0.49)	0.73 (0.44)	0.85 (0.61)	0.37 (0.24)
Germinated Conidia (%)	0.08 (0.02)	0.30 (0.11)	0.07 (0.02)	0.07 (0.02)	0.32 (0.12)	0.20 (0.08)
Number of germinated cells per conidium	0.27 (0.09)	0.51 (0.19)	0.17 (0.07)	0.26 (0.09)	0.51 (0.17)	0.38 (0.21)
Number of germinating tubes (50 conidia)	0.28 (0.09)	0.63 (0.21)	0.22 (0.08)	0.26 (0.10)	0.58 (0.21)	0.40 (0.22)
Size of conidia						
Length	0.13 (0.07)	0.63 (0.30)	0.08 (0.07)	0.13 (0.07)	0.17 (0.08)	0.03 (0.06)
Width	0.22 (0.11)	0.61 (0.29)	0.12 (0.09)	0.22 (0.12)	0.18 (0.09)	0.09 (0.16)
Canker size						
Length (mm)	0.52 (0.33)	0.63 (0.30)	0.52 (0.38)	0.48 (0.31)	0.62 (0.32)	0.30 (0.22)
Width (mm)	0.44 (0.25)	0.61 (0.29)	0.35 (0.27)	0.35 (0.25)	0.61 (0.31)	0.29 (0.23)
Genetic diversity						
Haplotype diversity	0.91	0.65	1.00	0.92	0.58	1.00
Gene diversity	0.98	0.88	1.00	0.98	0.87	1.00
Expected heterozygosity	0.41	0.30	0.35	0.41	0.31	0.20
Nucleotide diversity	0.076	0.065	0.075	0.113	0.082	0.027

Table 6. Phenotypic plasticity index (Plv = max mean – min mean / max mean) and coefficient of variation (in brackets) of all the assayed traits calculated for the geographic *S. cardinale* populations and the genetic sub-populations. Genetic diversity was evaluated for the populations and sub-populations of the fungus using three different indices: Haplotype diversity; gene diversity and gene diversity as expected heterozygosity.

However, one of the best option overseas is the use of resistant cultivars

Seiridium cardinale is listed as present in Europe and in California so no regulations apply, however virulence of California isolates is greater and resistant cypresses to the strains now present in the Meditterreanean, may be susceptible to new strains, this nullifying 30 years of research necessary to develop such cultivars

Sporulation: sporulation in the short period does not increase in either Med1 or Med2, suggesting lack of competition in the new range in the Mediterranean Basin

Longer term sporulation increases, as expected due to fact that it is linked to transmission. However it appears that increased sporulation is not easily evolvable, as it only evolves in Med2

Ca and Med populations remain distinct not only genetically but also

1- Ability to grow at extreme temperatures is lost in med populations (and not regained in MED 2).

2- Virulence of Med populations is lower than that of CA Populations. This is an advantage as the pathogen does not wipe out all of its hosts

“Emergent diseases”:

2: environmental changes

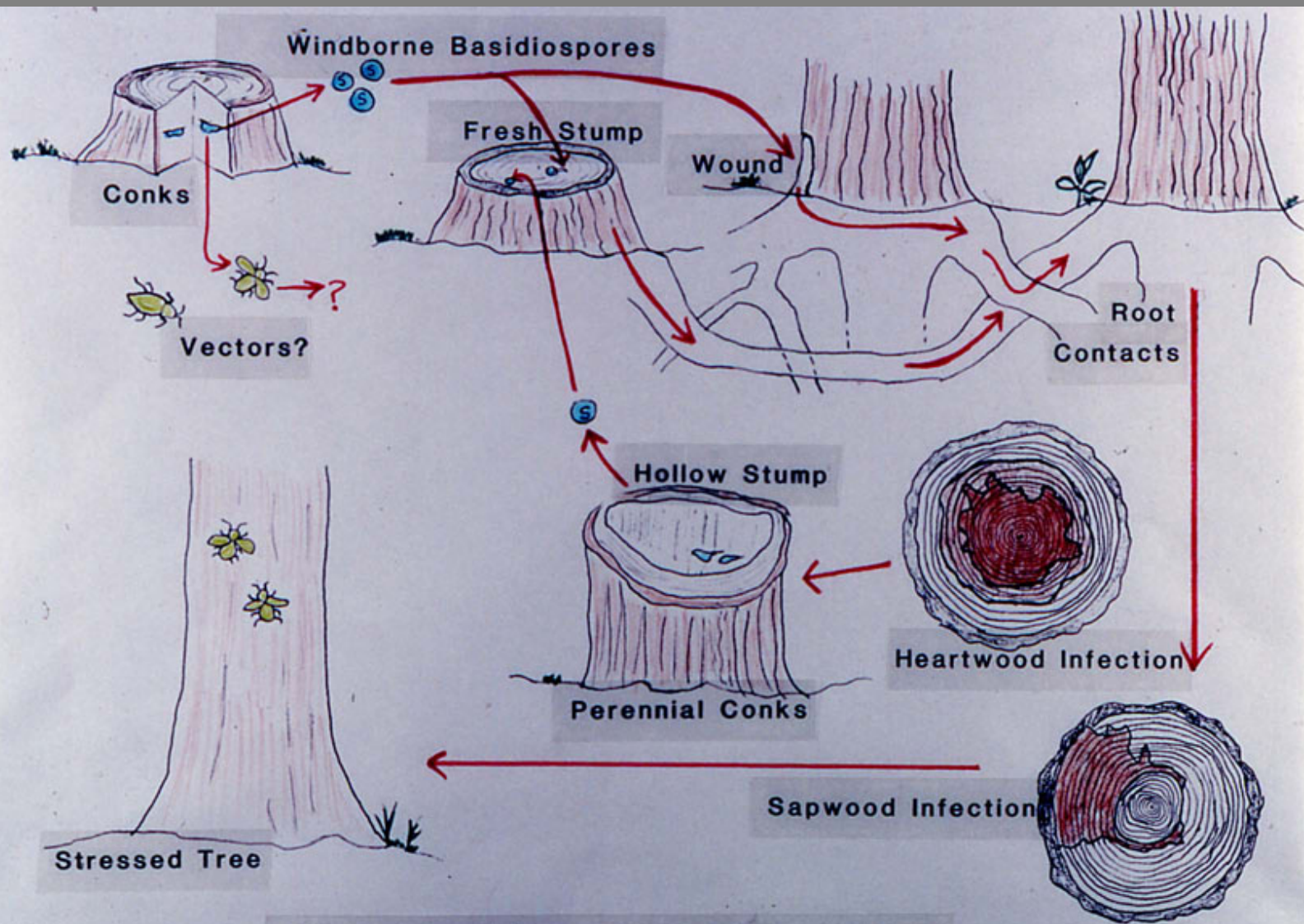
- **Forestry and intensive forest use:**
 - timber production**
 - tree felling and creation of stumps**
 - fire exclusion and increase in density**
 - oversimplified forest composition**
 - changes in forest composition**
 - changes in forest structure**

Heterobasidion root disease

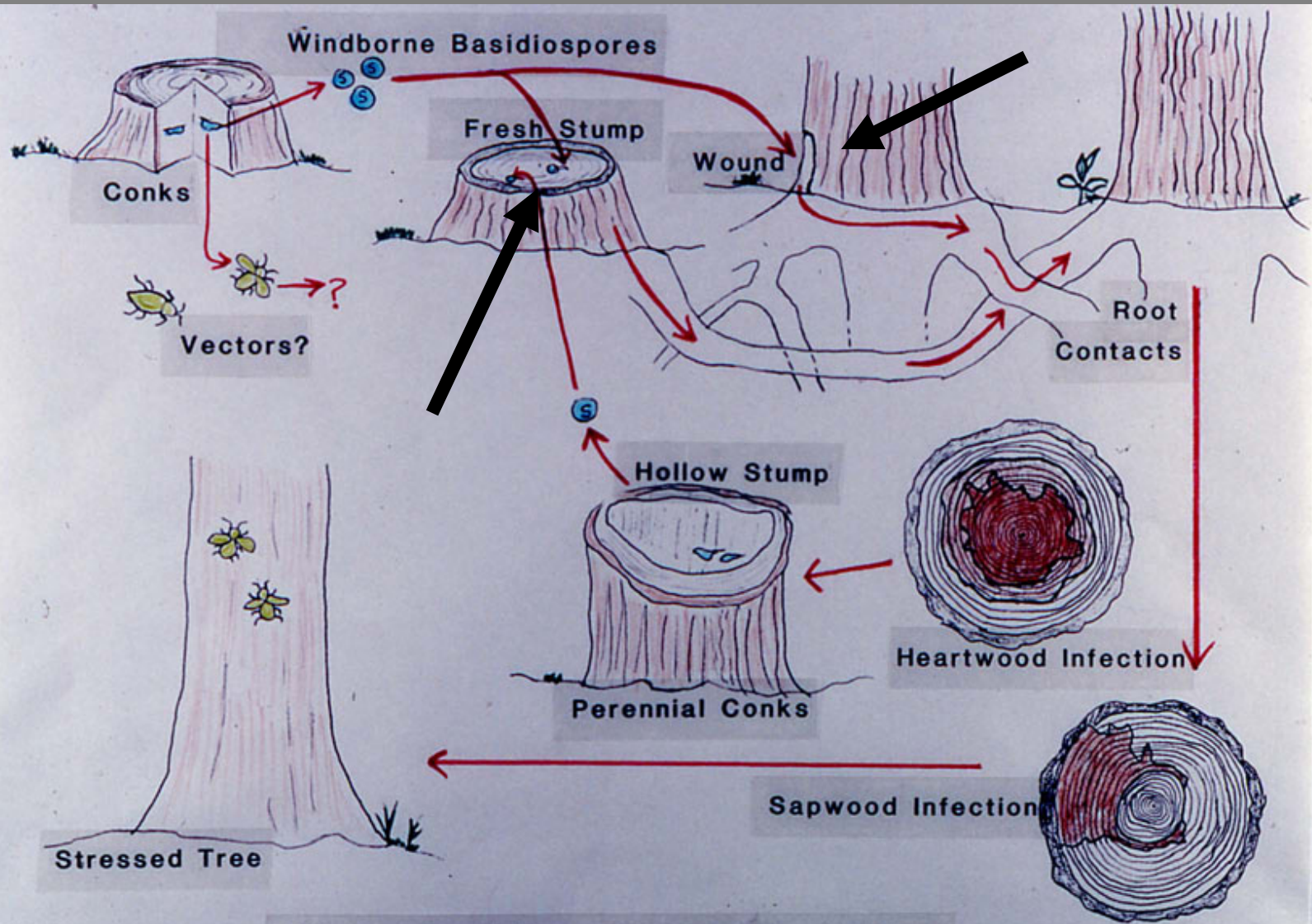
- *Heterobasidion* (a bracket or shelf mushroom) infects trees through wounds and stumps, then it spreads through the roots to neighboring trees
- With tree felling, stumps and wounds are created, suddenly exponentially increasing infection levels

Heterobasidion shelf fruit-body





Heterobasidion annosum* in *Abies



Heterobasidion annosum in *Abies*

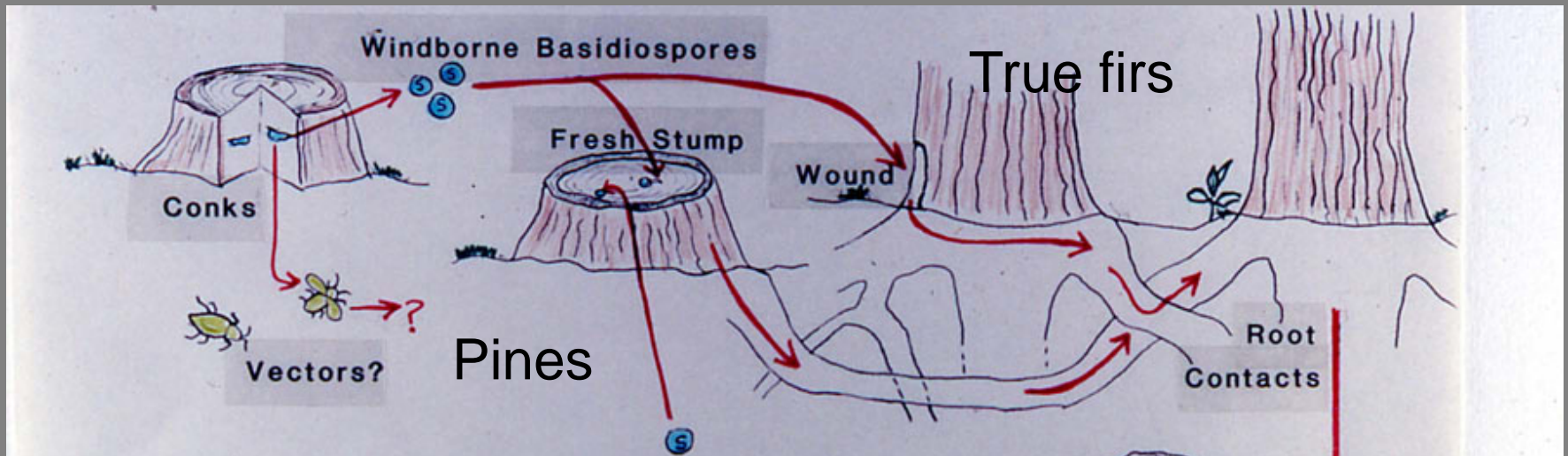


**Stump creation and subsequent infection
by *Heterobasidion annosum***



Use of molecular genetics:

- Differentiate *Heterobasidion* on fir/sequoias (*H. occidentale*) from that on pine/junipers (*H. irregulare*)
- Show that airborne meiospores are responsible for most infection of *Heterobasidion*
- Show that in pines most infections start on stumps and that in true firs most infections on wounds



Each spore is a genetically different individual:

In pines we found the same genetic individual in stumps and adjacent trees indicating direct contagion between the two

In true firs and true firs/sequoias we find same individual in adjacent standing trees indicating infection not linked to stumps but to wounds on standing trees

CONCLUSIONS:

- Logging activities increase *Heterobasidion* infection because of stump creation in pines and because of wounding in true firs sequoias
- We have shown that in pine stumps *H. irregulare* and *H. occidentale* can both be present and create a new hybrid entity
- We have shown that in the past these hybridization events have lead to sharing of genes among these two species (Horizontal gene transfers)

Once Heterobasidion is established at high frequency

- Significant loss in timber value
- Dangerous situations (campsites)
- Mortality of valuable species such as sequoia
- With insects and pollution it makes trees more susceptible to mortality
- Can make fires more destructive

Sequoia National Park



Annosus root disease in giant sequoia



Ponderosa pine



Incense cedar





Yosemite Lodge complex 1972

cabin crushed by tree with
rotted roots

since 1973
7 fatalities

19 serious injuries
Over \$1M property damage



Yosemite Lodge 1975 Root disease centers outlined



Yosemite Lodge 1997 Root disease centers outlined



Many gaps with very little regeneration and have not closed in



Change in gap area 1972-1999

Year	Area in gaps (m ²)	Percent in gaps
1972	6125	3.5
1999	53,981	31

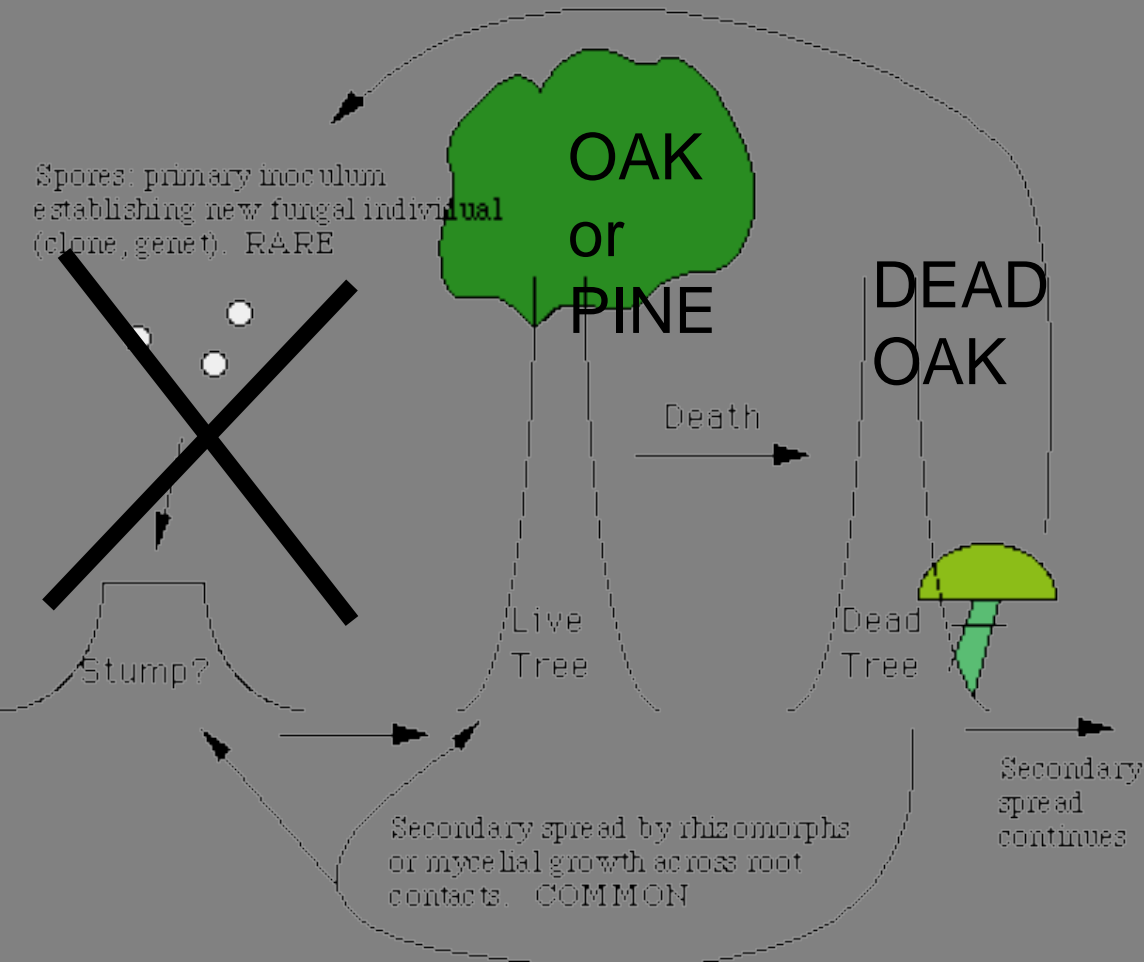
Armillaria root diseases

- *Armillaria*, the honey mushroom, normally infects the roots of trees. It can be a saprobe and a pathogen and is common amongst oaks
- If woodland composition shifts to pine/oak, pines become the target of attacks and gaps in canopy enlarge over time. Stress (e.g. flooding) exacerbates susceptibility

Clusters of *Armillaria*



How Does it Infect?



Two means of dispersal to other trees:

1. Mycelium can grow through direct root contacts and grafts with uninfected trees.
2. Rhizomorphs can grow through soil to contact uninfected trees.

What are Rhizomorphs?

- ...“conglomerations of differentiated parallel hyphae with a protective melanized black rind on the outside.”



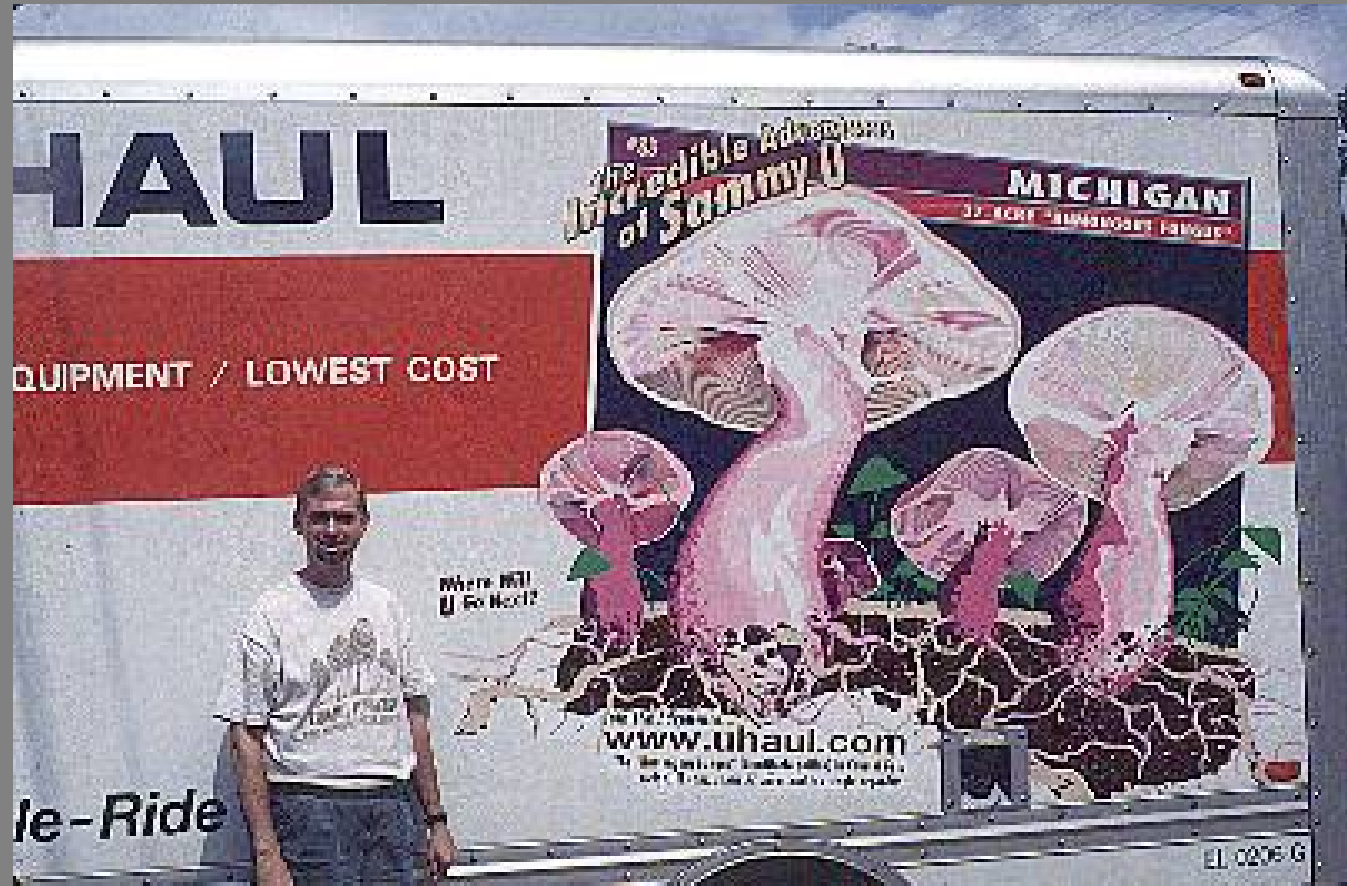
SOURCE: <http://www.nifg.org.uk/armillaria.htm>

- Rhizomorphs are able to transport food and nutrients long distances which allows the fungus to grow through nutrient poor areas located between large food sources such as stumps.

SOURCE: http://botit.botany.wisc.edu/toms_fungi/apr2002.html

Humongous Fungus

It's One of
U-HAUL's
“Bizarre
Roadside
Attractions”



http://botit.botany.wisc.edu/toms_fungi/apr2002.html

CONCLUSIONS

Human activities shifting from oak woodlands to mixed oak-pine lead to large mortality gaps in pines around oaks if honey mushroom is present

CHANGING SPECIES
COMPOSITION LEADS TO
SEVERE DISEASE

Many gaps with very little regeneration and have not closed in



How can people transport pathogens

- By transporting plants and plant parts
 - Crops, and seeds
 - Raw food
 - Ornamental plants
- Untreated lumber
- Soil
- Insects vectoring fungi
- Military activity



Invasion of an exotic root pathogen of forest trees: the case of *Heterobasidion annosum*

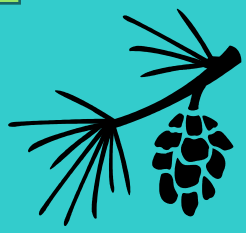
P. Gonthier¹, R. Linzer², G. Nicolotti¹ & M. Garbelotto²

¹ Di.Va.P.R.A., University of Torino, Italy

² E.S.P.M., University of California at Berkeley, USA

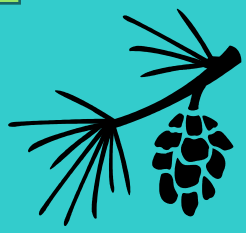


Eighth International Mycological Congress, Cairns, Australia, 20-25 August 2006



The *Heterobasidion annosum* (Fr.) Bref. species complex

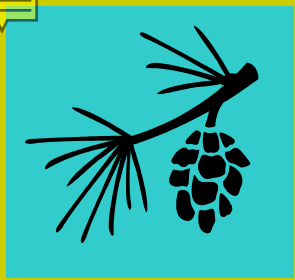




Italian Stone Pine (*Pinus pinea* L.) mortality centers in Castelporziano (Rome)

2002

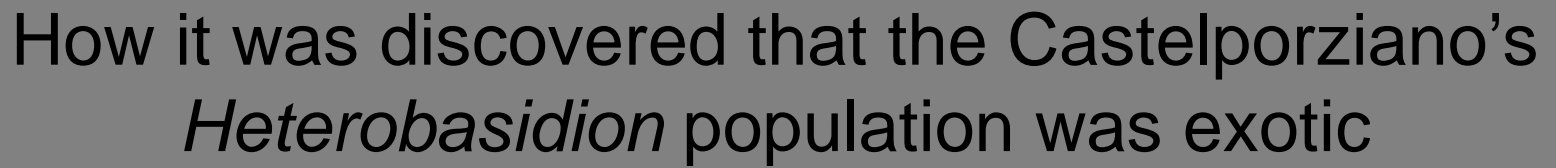




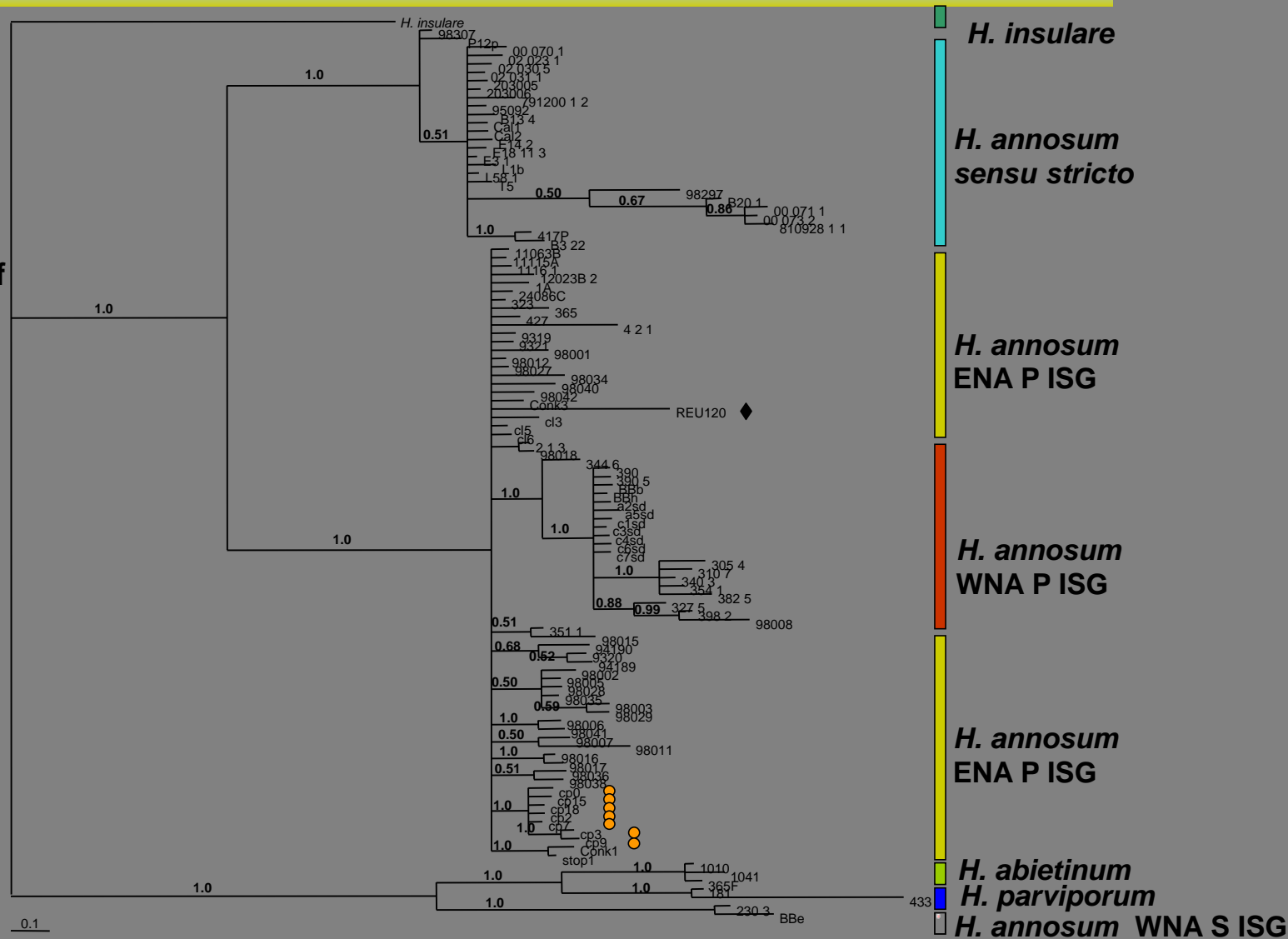
Basidiomata of *Heterobasidion* sp. on pine stumps within Castelporziano's pine mortality centers

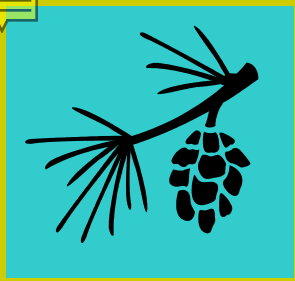
2002





Gonthier *et al.*, 2004
Warner *et al.*, 2005
Linzer *et al.*, in prep.



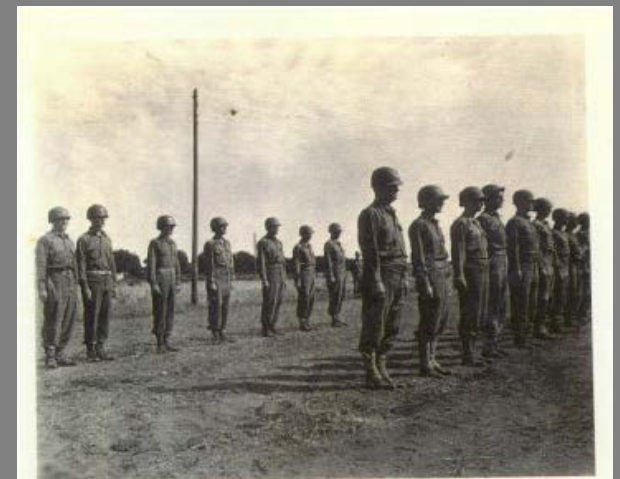


How was *Heterobasidion* introduced from Eastern North America?

The Estate of Castelporziano has been closed to the public since 1562, and...
is comprised of an exclusively native Italian flora (Manes *et al.*, 1997)

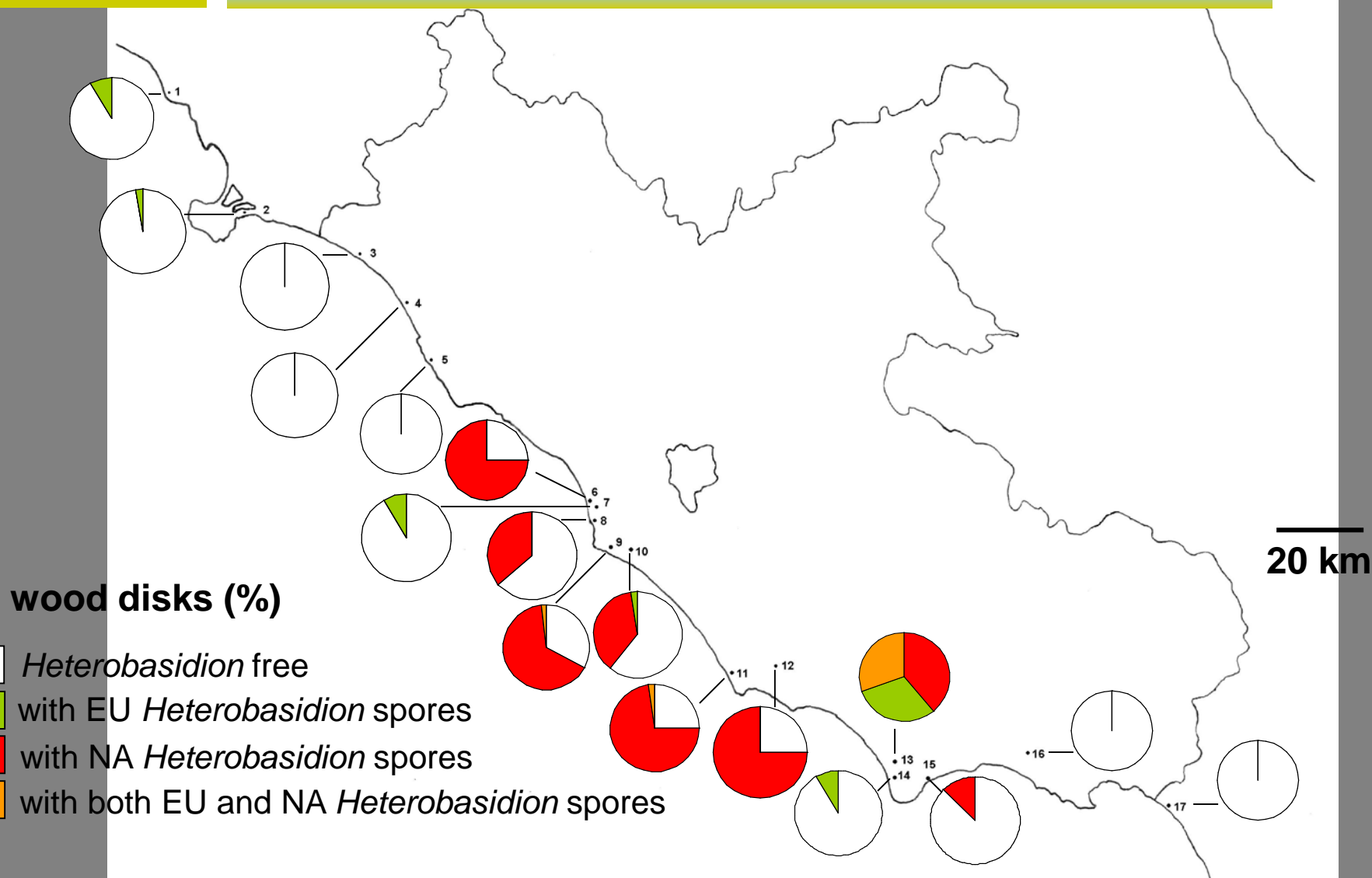
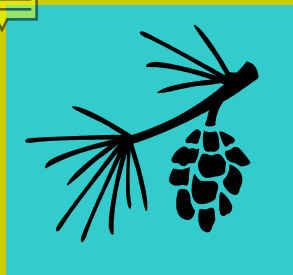


June 5th - July 10th, 1944
training and resting camp



337th Regiment Review Castelporziano

Current distribution of the exotic pathogen



Why exotic outcompetes native

- It is equally pathogenic (can't always blame lack of coevolution)
- It produces a lot more spores (transmission)
- It is a better saprobe (establishment)
- It can colonize oak stands

Hybridization: why care?

- Hybrid pathogens can change hosts
- Hybridization allows for gene introgression and can increase virulence
- We have documented that about 17% of alleles are completely new due to recombination: what will these alleles be able to do