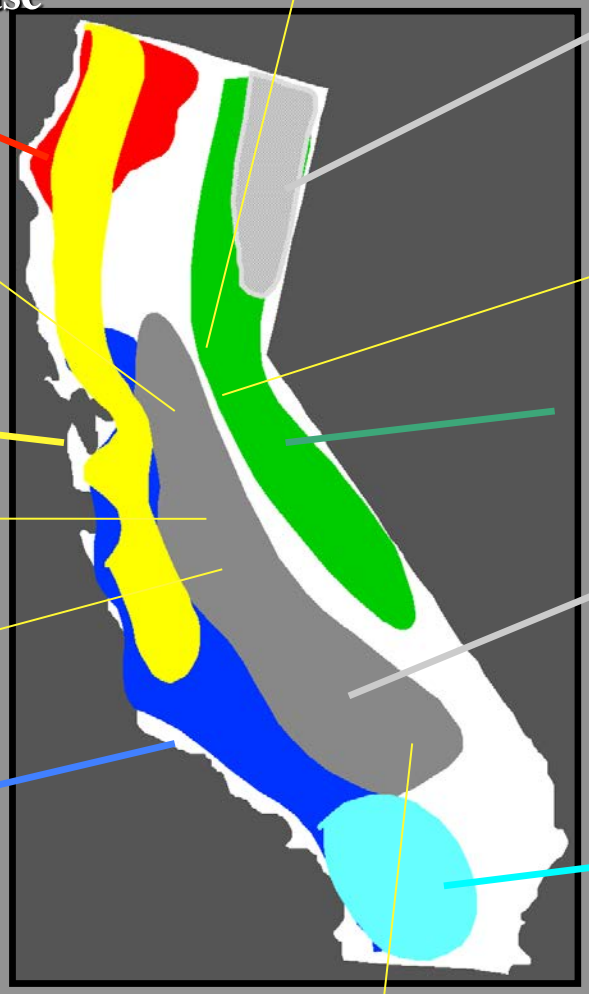
California invaded: 1849 A.D.



Xylella scorch of maples 2000s

Port Orford Cedar Root Disease 1950s

Expansion of root pathogens Post 1880s

Root canker of Pacific Madrone and Bay laurel (70s)

Manzanita die-back 2004

Sudden Oak Death 1990s

White pine blister rust 1930s

Cypress canker 20s

Dutch Elm Disease 1960s

Colored canker of sycamore 70s

Pitch canker disease 1980s

Oak root canker 2000

1000 canker disease of walnuts 2001

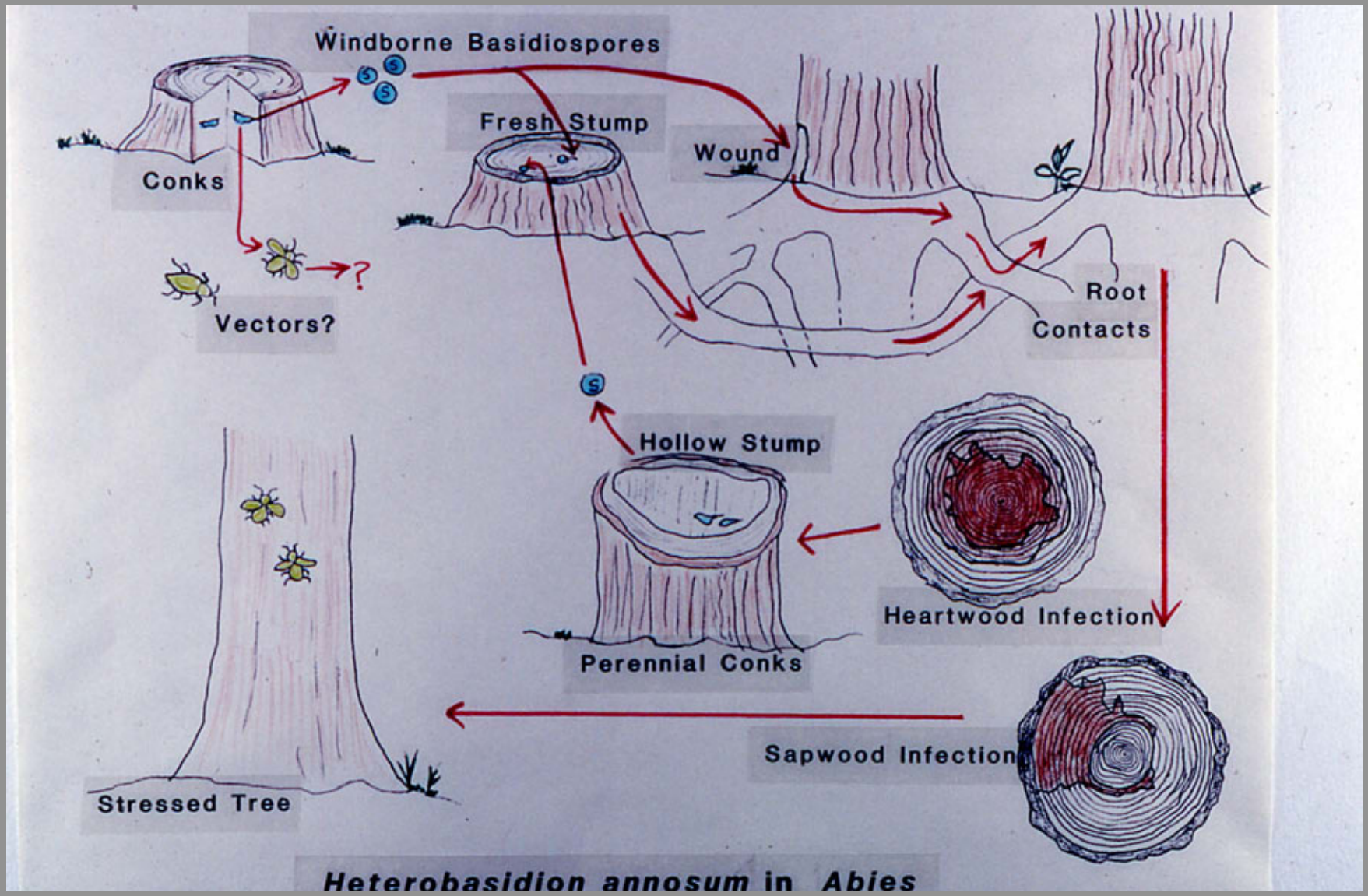
“Emergent diseases”: 2: environmental changes

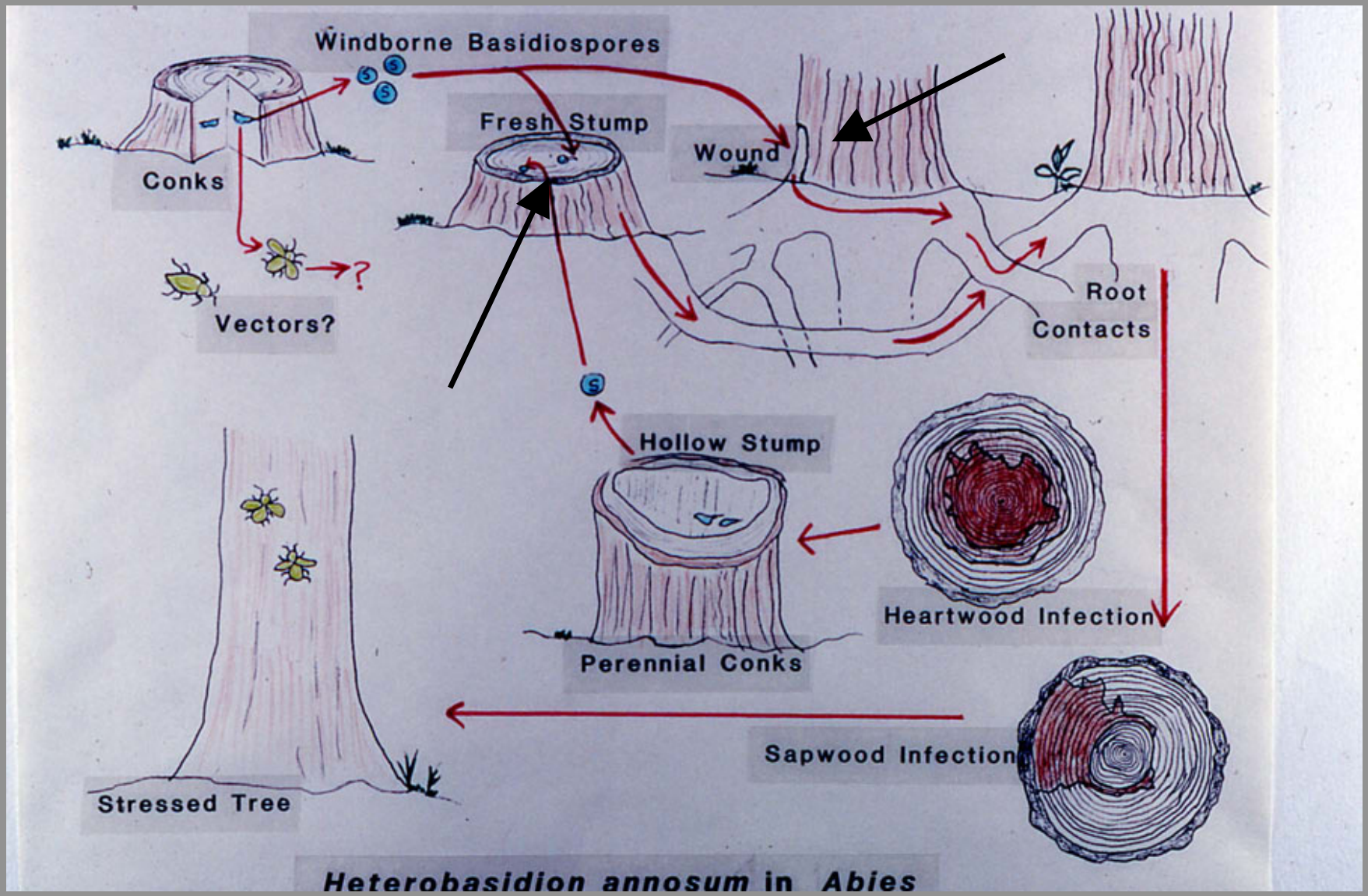
- Forestry and intensive forest use:
  - timber production
  - tree felling and creation of stumps
  - fire exclusion and increase in density
  - oversimplified forest composition
  - changes in forest composition
  - changes in forest structure

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

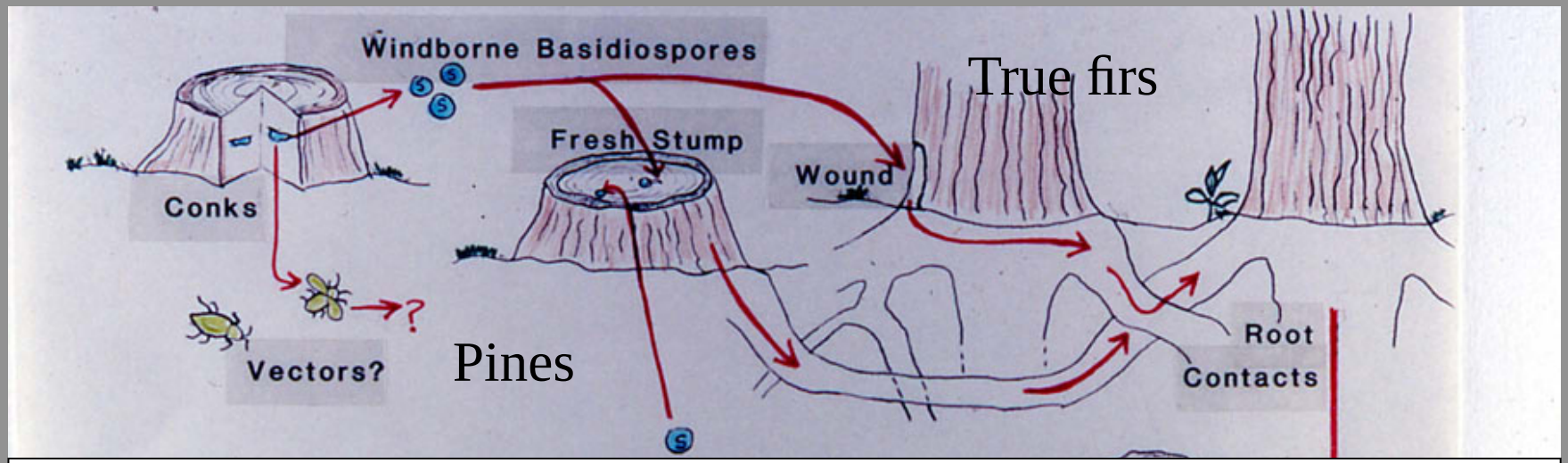

Windborne Basidiospores
Fresh Stump
Wound
Conks
Vectors?
?
Root
Contacts
Hollow Stump
Heartwood Infection
Perennial Conks
Sapwood Infection
Stressed Tree
Heterobasidion annosum in Abies


Windborne Basidiospores
Fresh Stump
Wound
Conks
Vectors?
?
Root
Contacts
Hollow Stump
Heartwood Infection
Perennial Conks
Sapwood Infection
Stressed Tree
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

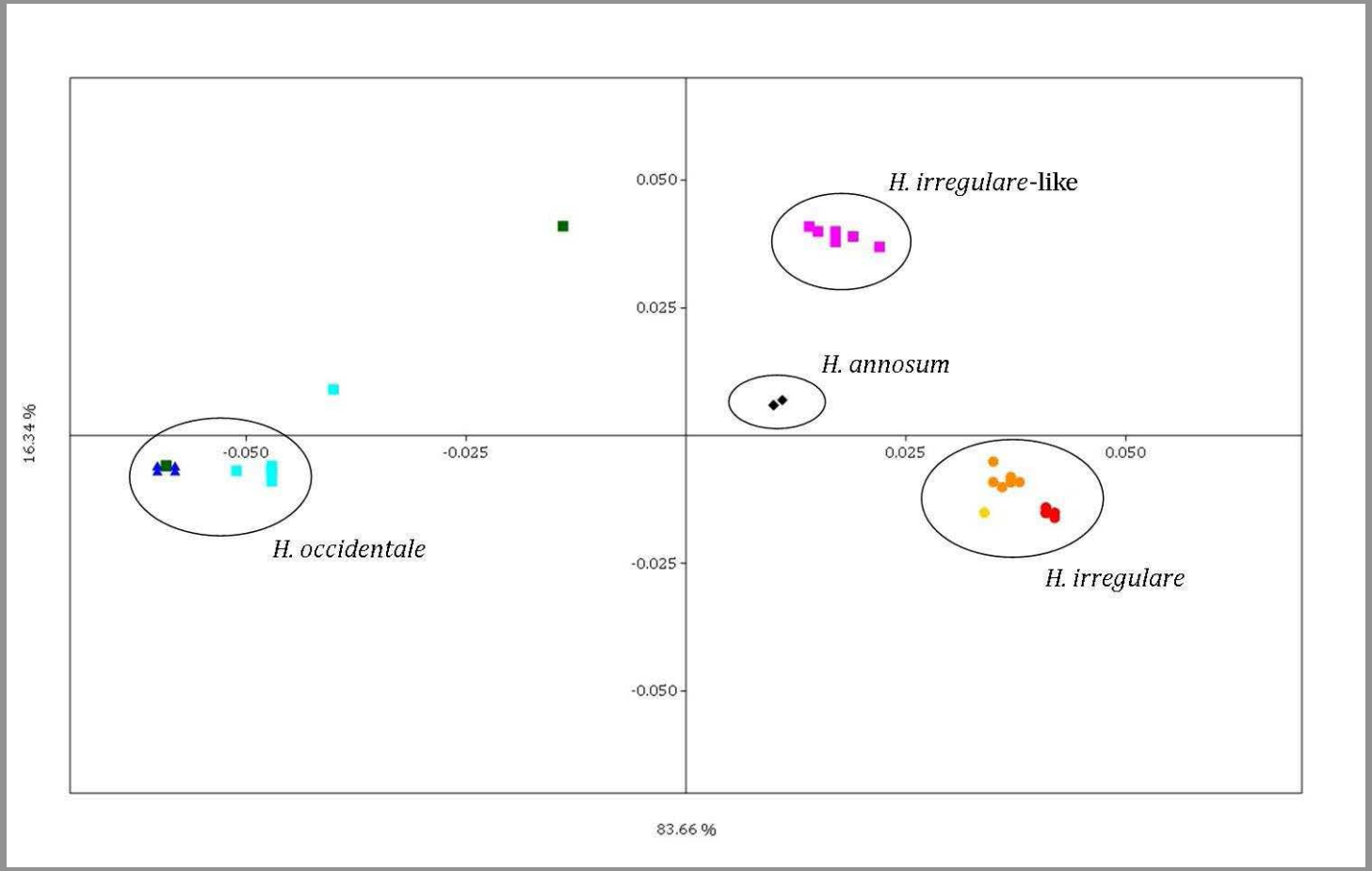
In true firs and true firs/sequoias we find same individual in adjacent standing trees indicating infection not linked to stumps but to wounds on standing trees

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)

New disease of Alpine larch

Fig. 1. Symptomatic larches (left) and a *Heterobasidion* basidiocarp (top).

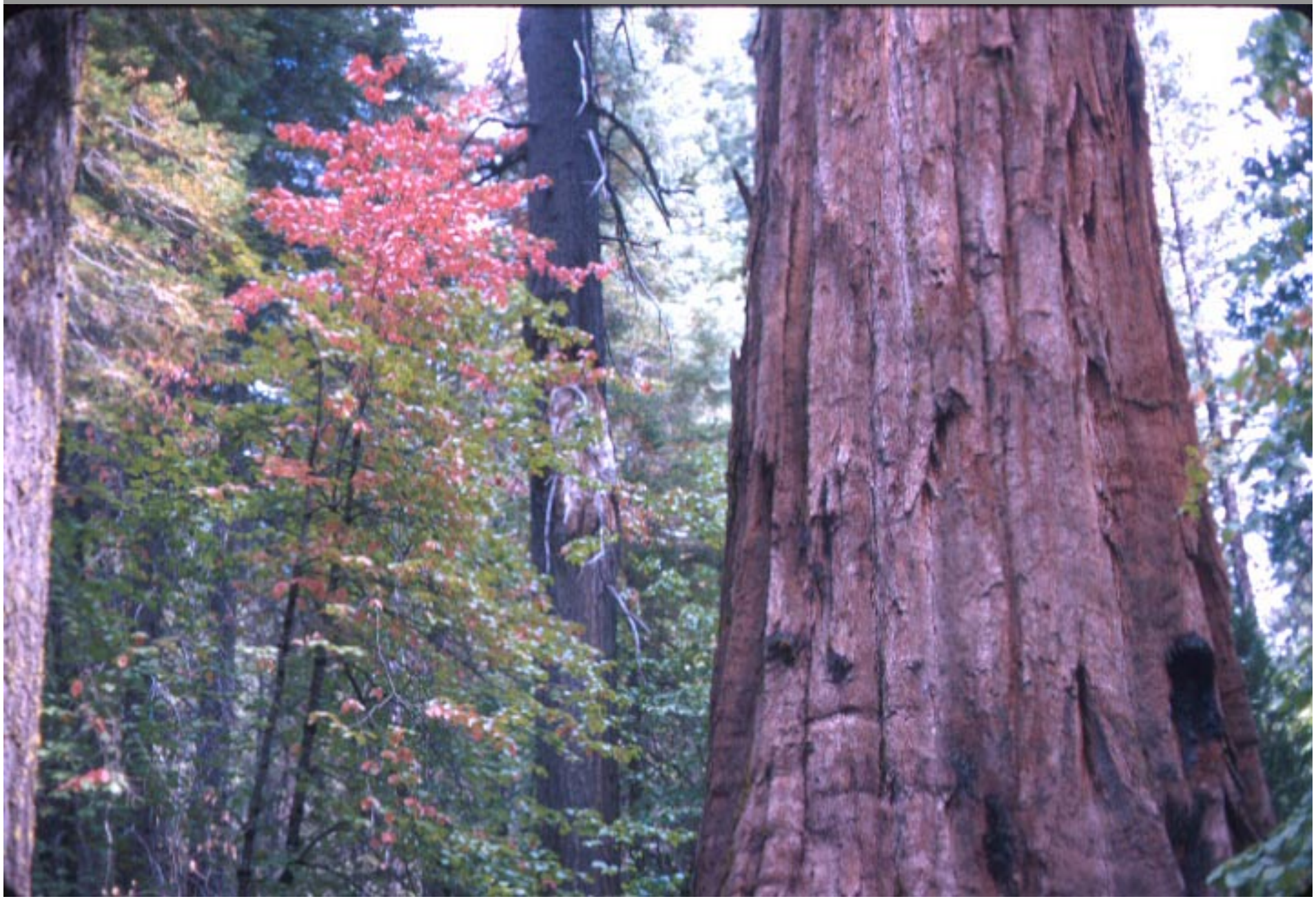


H. Irregulare-like is a new species generated by hybridization between *H. irregulare* and *H. occidentale* on a host that could be infected by both: alpine larch!!!

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

Oomycota

- Belong to a kingdom that includes kelp and diatomes
- Kingdom used to be called Chromista (brown algae), it is now the Straminipila
- It includes many important plant pathogens:
  - *Peronospora*, *Plasmopara*: mostly aerial
  - *Pythium*: mostly soilborne organisms
  - *Phytophthora*: mixed biology

Blue mold of tobacco caused by *Peronospora tabacina*

- Ability to travel aerially for hundreds of kilometers from Caribbean to Southern US
- Ability to predict arrival of inoculum based on weather pattern
- Some species capable of over-wintering in buds

Oomycetes are <u>not</u> fungi

- Cellulose in cell wall
- Ploidy is 2n
- Result of sexual activity is oospore (2n)
- Meiosis, somatogamy, caryogamy all occur at the same time

- Water adapted biology, flagellate phase
- No septa, holocoenocytic hyphae

- Chitin in cell wall
- Ploidy is n, or n+n
- Result of sexual activity is a spore n
- Meiosis, somatogamy,caryogamy are usually interupted by vegetative (somatic phase)
- Better adapted for aerial transmission
- Septate hyphae

Important structures

- **Sporangia**: size, shape, L:B, papillate or not, deciduous or not
- **Stalks**: length
- **Zoospores**. **Encysted zoospores**
- **Chlamydospores**: how are they carried (lateral vs. terminal), size, color, ornamentation
- **Oospores** (how male and female cross)
- **Hyphae:** swellings present or absent, linear or tormented
- **Colony morphology:** appressed vs aerial, fast-growing vs. slow-growing

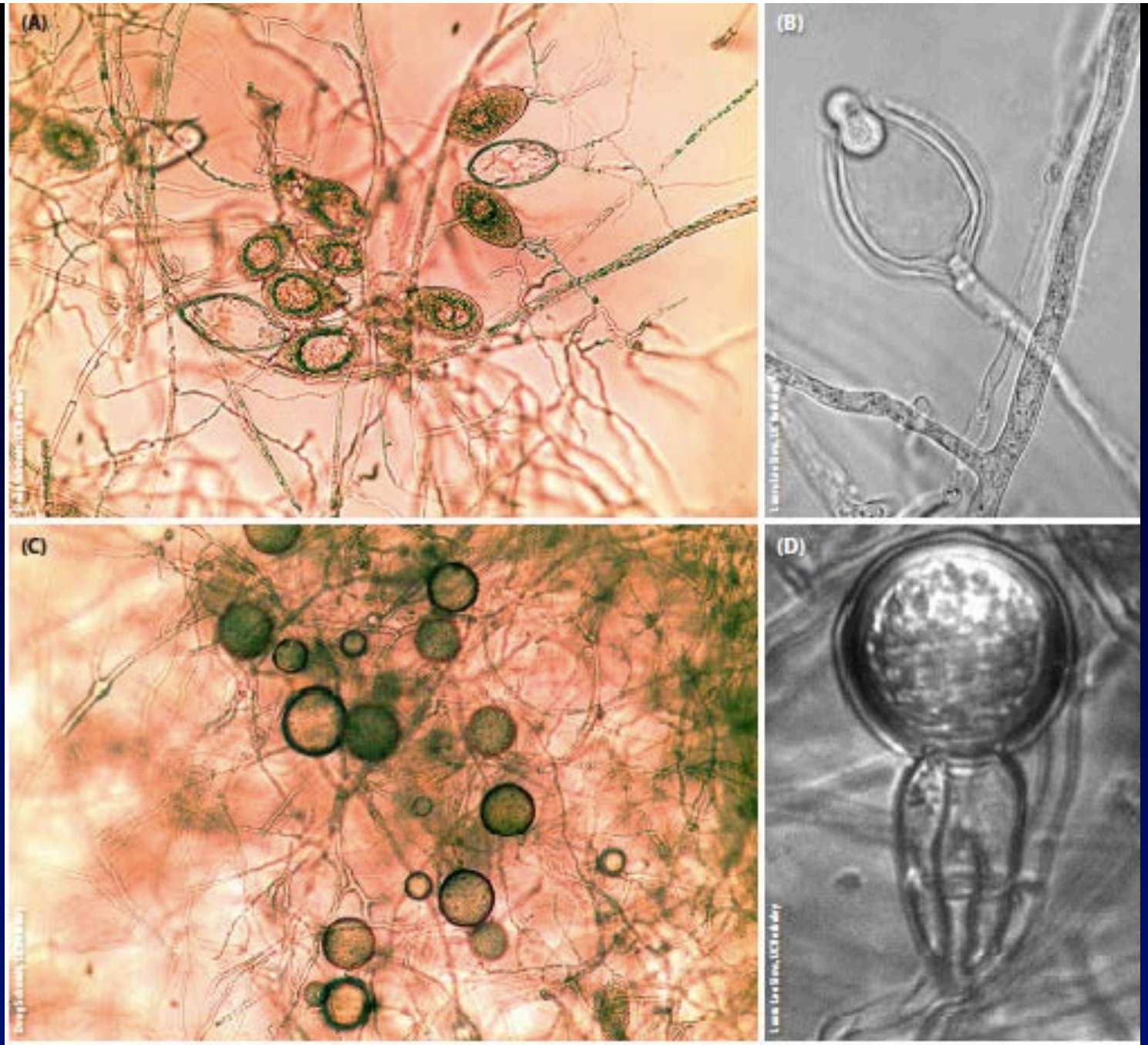


FIG. 1. Micrographs (300x magnification) of (A) sporangia of *Phytophthora ramorum*, (B) a zoospore exiting a sporangium of *Phytophthora* taxon oaksoil, (C) chlamydospores of *Phytophthora ramorum*, and (D) an oospore of *Phytophthora alni* subspecies *uniformis*.

Phytophthora

- Some important plant pathogens, with very well known history
  - *Phytophthora infestans* and the Irish potato famine
  - *Phytopthora cinnamomi* and the Jarrah dieback in Australia

The Irish Potato Famine

- From 1845 to 1850
- *Phytophthora infestans* arrived in Ireland causing Late Blight of potato
- Resulted in the death of 750,000
- Emigration of over 2 million, mainly to the United States.

WOMAN BEGGING AT CLONAKILTY.

Phytophthora: "plant destructor"

- Best known pathogen whose long-distance transport linked to agriculture.
  - *Infected root-stocks*
  - *Infested soil*
  - *Infected plants*

150+ species of *Phytophthora*

- 60 until a few years ago, research accelerated, especially by molecular analyses
- Differentiated on basis of:
  - Type of sexual intercourse
  - Type of sexual activity
  - Number of hosts
  - Ideal temperature
  - Type of biology
  - Evolutionary history (Waterhouse-Cooke)

Type of sexual strategy

Homothallic species, will produce both oogonia and antheridia and mate by themselves (hermaphrodite), low genetic variability. Strong inbreeding. Because they prolifically produce oospores can survive in harsher climates (interior CA, high altitude, etc.)

Heterothallic species need two individuals with different MATING TYPES. Normally defined as A1 and A2. Out-crossing species.

Type of sexual strategy

If species is exotic, expectations are:

-Often one mating type only, or mating types introduced at different times.

-Low genotypic diversity, prevalence of clonal lineages

-If species is homothallic expectation is that all individuals will be similar, because there has been no time for genetic differentiation

Type of sexual strategy

Why should we care about sex ?

-Ability to create new alleles, better potential of adaptation to new conditions

-Ability to exchange genes with other individuals, if gene pool is large, it can be a great advantage

-For instance ability to overcome the fungicide metalaxyl happened when A1 and A2 of *P. infestans* got together and reproduced

- Sexual structures may be hardier

Number of hosts

- Single hosts, specialized: *P. sojae*, *P. lateralis*
- Multiple hosts, generalists: *P. cinnamomi* (3000 hosts!), *P. ramorum* (> 60)

Temperature

- **Optimal temperatures**: explain why many species are extremely seasonal, also correlated to area of original evolution of species.
  - Thermofilic spp. (summer species): *P. palmivora, P cinnamomi, P. citricola*
  - Psychrofilic spp. (winter species): *P syringae, P lateralis, P. hybernalis, P. ramorum*

- **Extreme temperatures**: ability to withstand extreme cold or heat. Normally depends on resting structures, and where they are produced.

  Species producing abundant chlamydospores within plant tissue will be more resilient

Type of biology

- **Waterborne, soilborne**
- Mostly root-infecting
- Can move from roots into root collar and stem
- Moves using water accumulations (floods) or streams
- Infested soil source of easy infection

- **Aerial**
- Infect leaves, twigs and branches
- Can move onto stems
- Moves using wind
- Need rainy conditions to infect (free water needs to accumulate)

Soil-Waterborne vs.Aerial

- *P. cinnamomi*
- *P. citricola (plurivora)*
- *P. cactorum*
- *P. quercina*
- *P. cambivora*
- *P. palmivora*
- *P. ramorum*
- *P. nemorosa*
- *P. pseudosyringae*
- *P syringae* (because of pruning)

Aerial species

- Recently discovered for forests: all characterized by deciduous sporangia
- Distance of spread depends on how dry sporangia are. If sporangia are dried they can go far
- True aerial will naturally infect aerial parts without need for root infections or tranmssion by tools
- Ability to rest in soil with resting structures is not lost!!, but epidemiological relevance not clear in nature
- Chemotaxis: ability to move towards susceptible host, may be lost in darkness

Soilborne, waterborne species

- Clear association with water: along streams, in areas that are temporarily flooded
- Ability to rest in soil with resting structures such as chlamydospores, oospores, but also encysted zoospores
- Chemotaxis: ability to move towards susceptible host, even in darkness

Phytophthora cinnamomi

- Oomycete (Kingdom Chromista, brown algae)
- Heterothallic (requires 2 mating types for sex)
- Soilborne pathogen- infects roots/stem collars
- Present in >67 countries
- Isolated from >1000 plant spp.
- Introduced pathogen to Australia in early 1900's

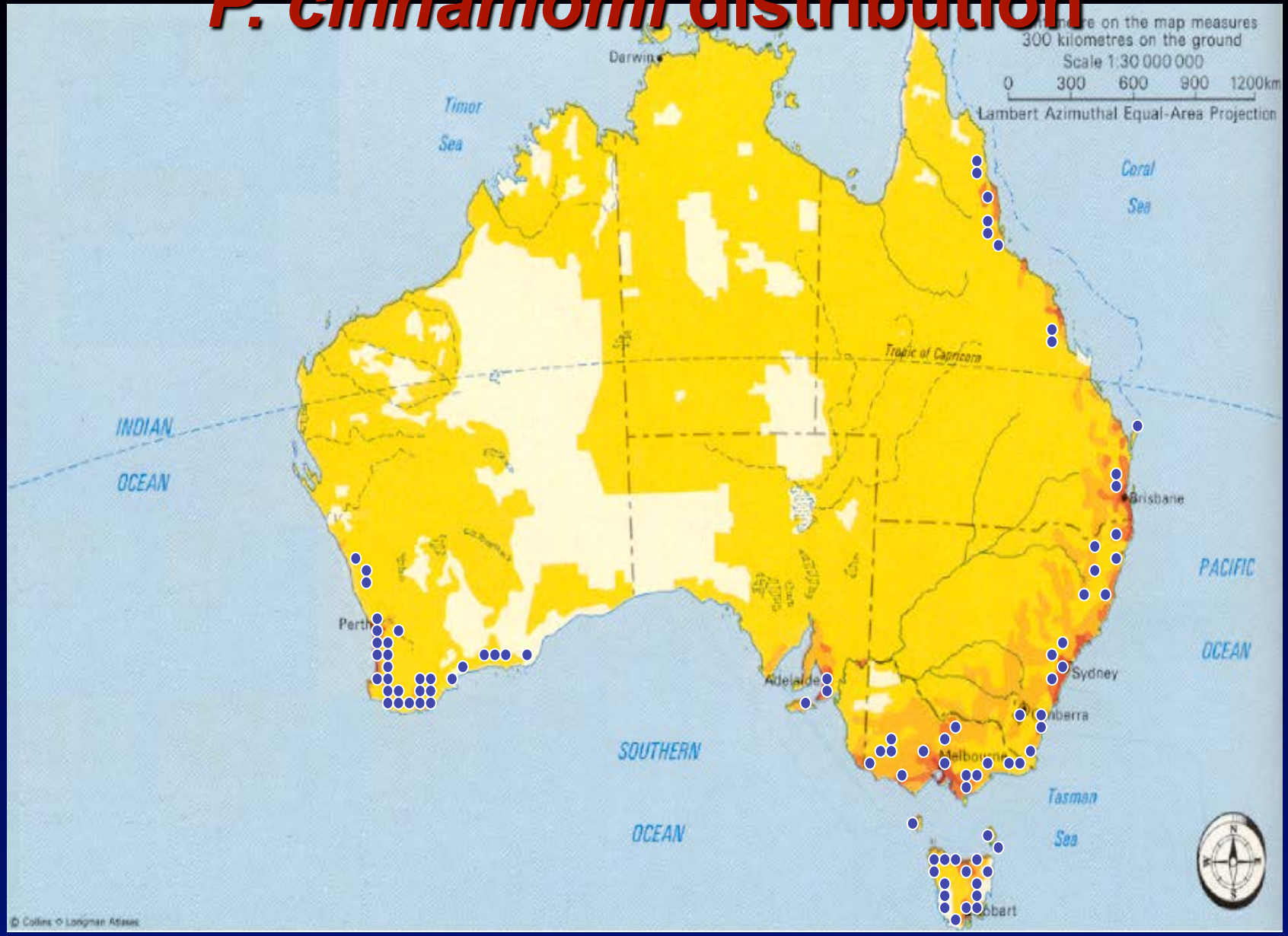
P. cinnamomi in Western Australia

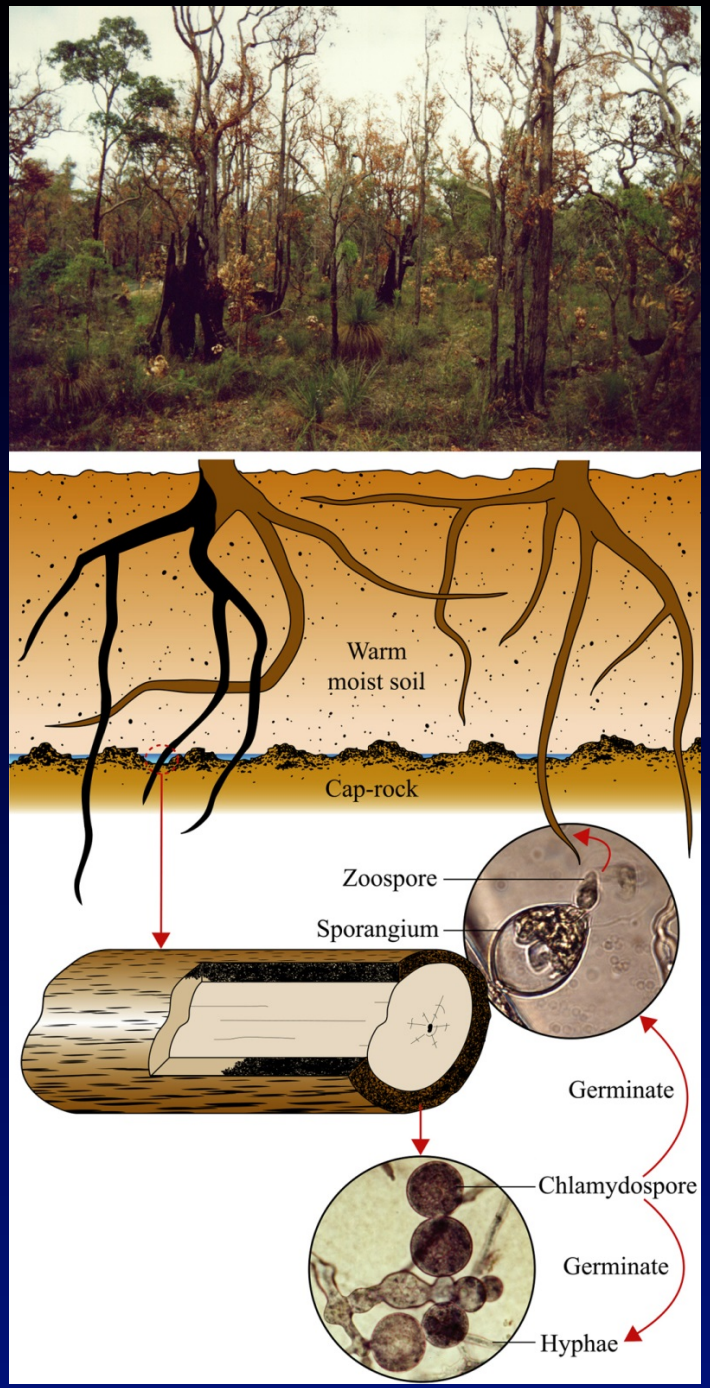
- Causes disease “jarrah dieback”
- 1921 first jarrah deaths; 1964 deaths shown to be caused by *P. cinnamomi*.
- Predominantly A2 mating type
- Three clonal lineages
- No sexual reproduction occurs

Impact Natural Ecosystems

- Between 8-9,000 plant species in south-west of Western Australia
- Approximately 2000 species are susceptible to *Phytophthora cinnamomi*
- Indirect effects of *P. cinnamomi* on plant and animal communities is unknown

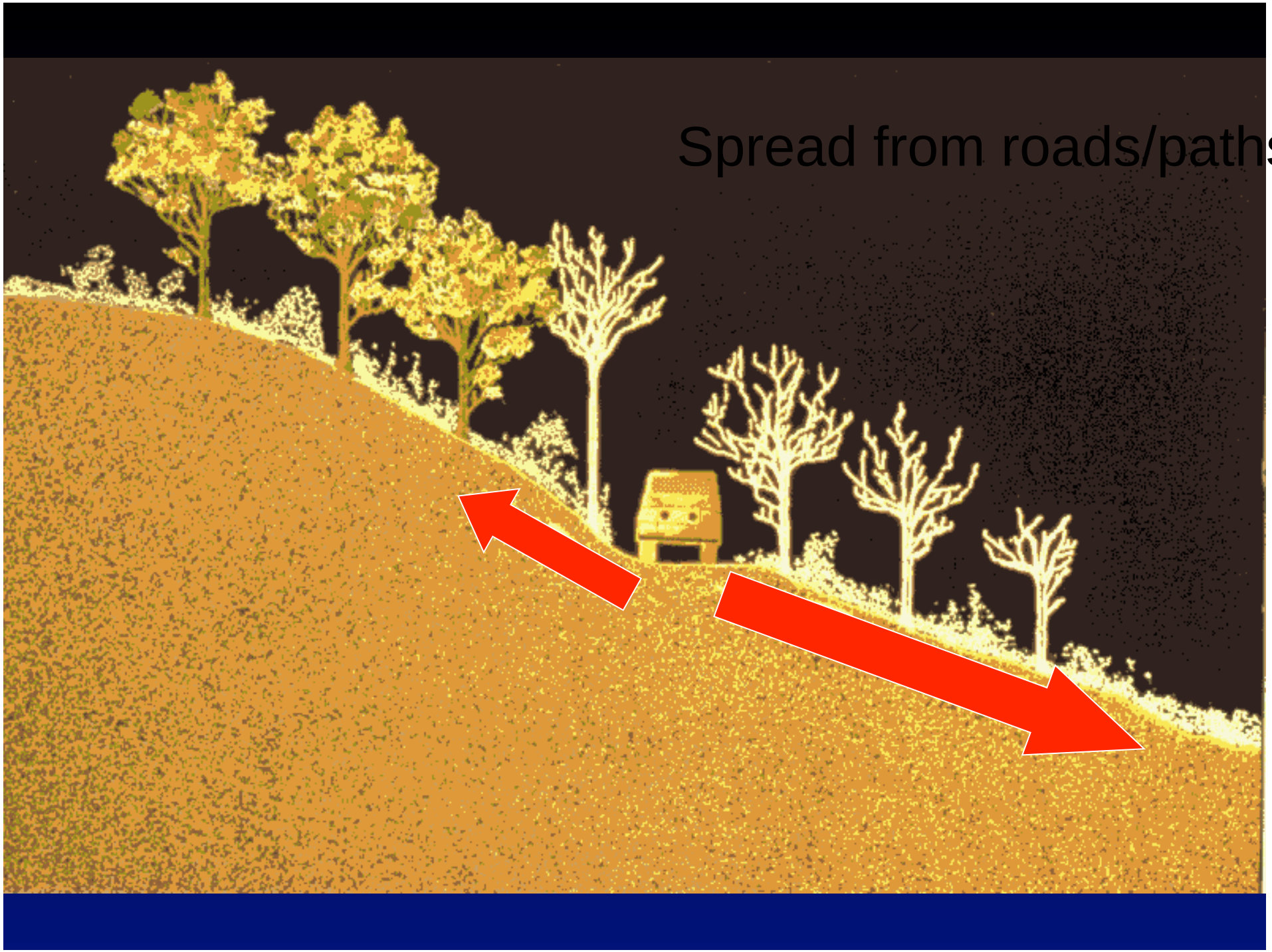
P. cinnamomi distribution



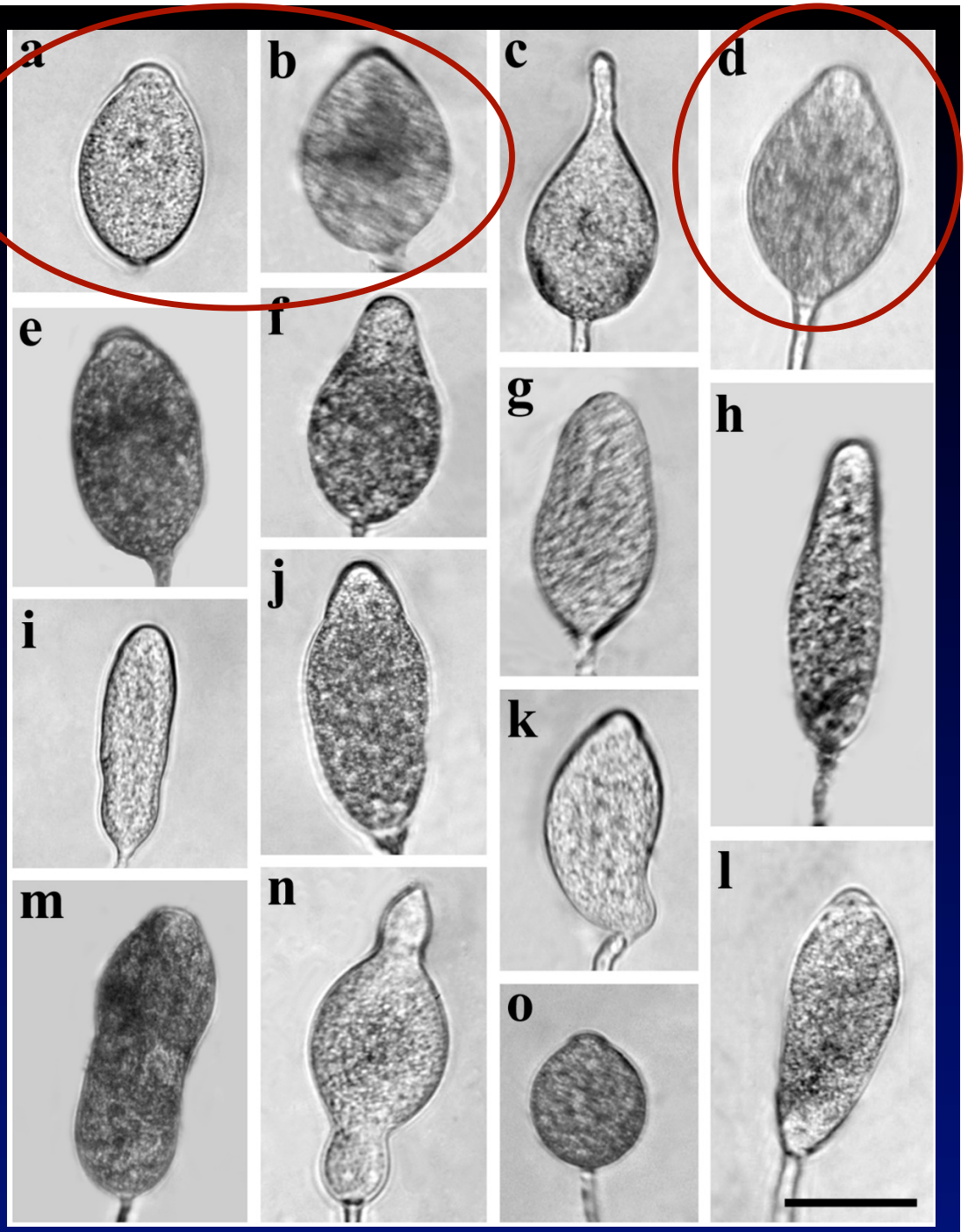

Warm
moist soil
Cap-rock
Zoospore
Sporangium
Germinate
Chlamydospore
Germinate
Hyphae

How is it spread?

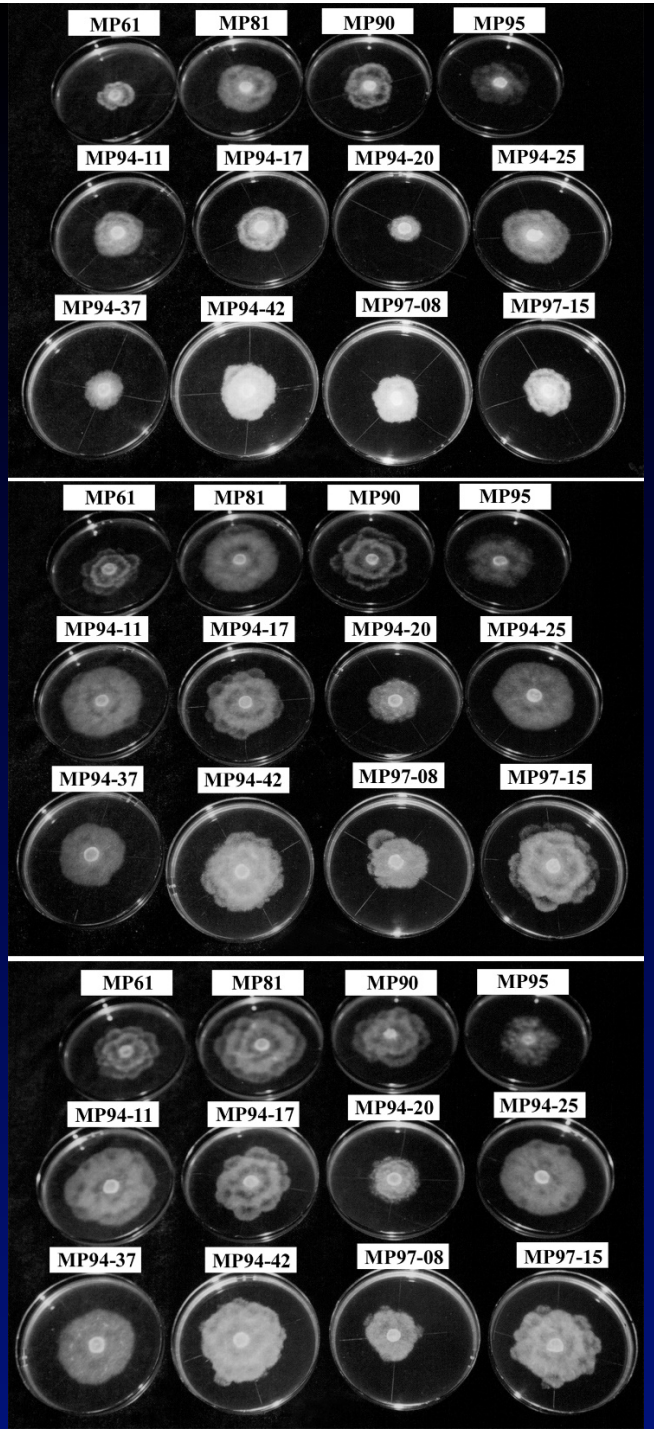
- Natural – root contact, free draining water (warm & moist; spring, summer & early autumn)
- Artificial – transport of infested soil (tyres, road making)
  – hikers (boots, tent pegs & toilet trowels)
  – planting infected nursery stock


Spread from roads/paths

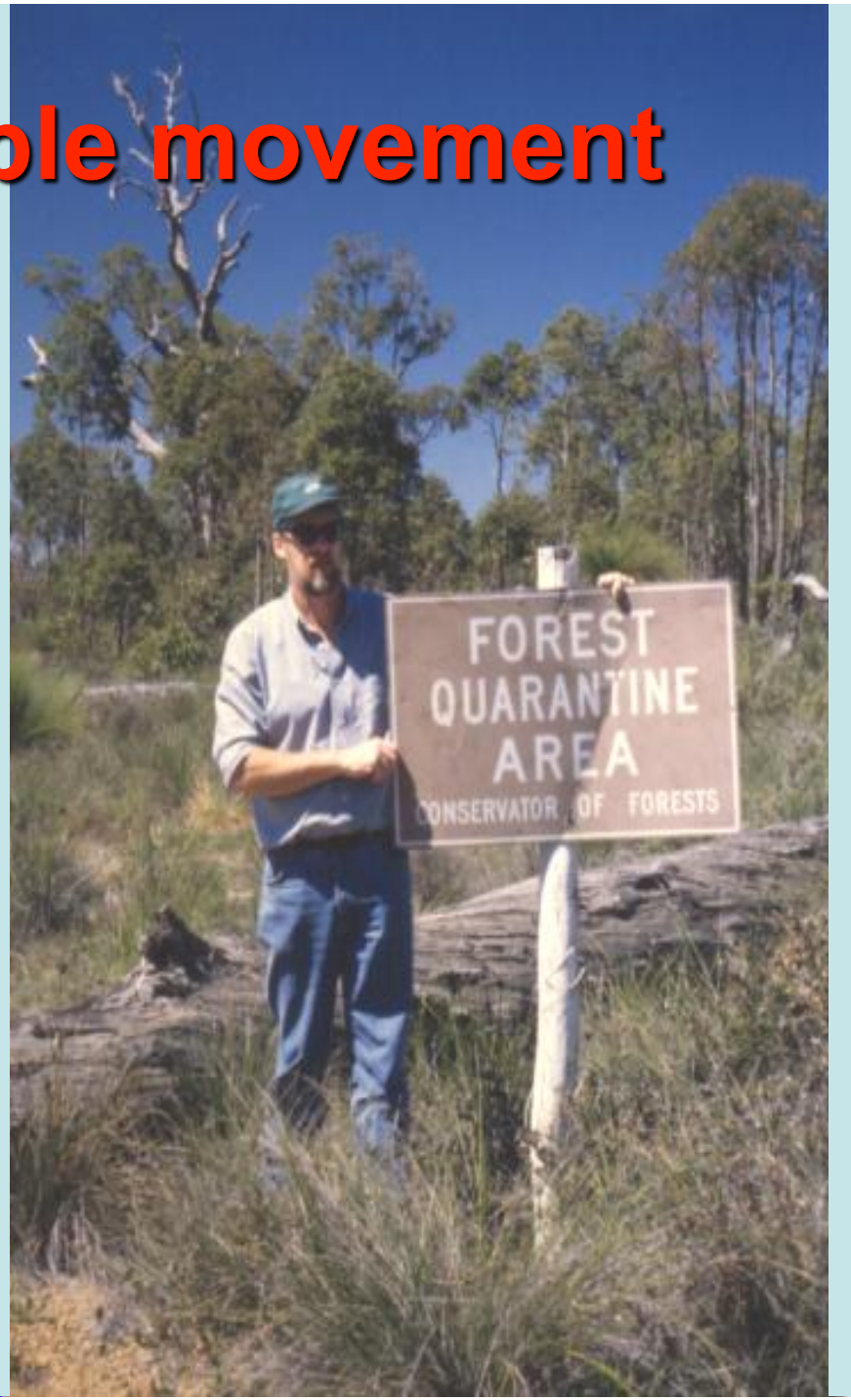
Typical
P. cinnamomi
sporangia



Variation in growth rate & colony morphology at different temperatures

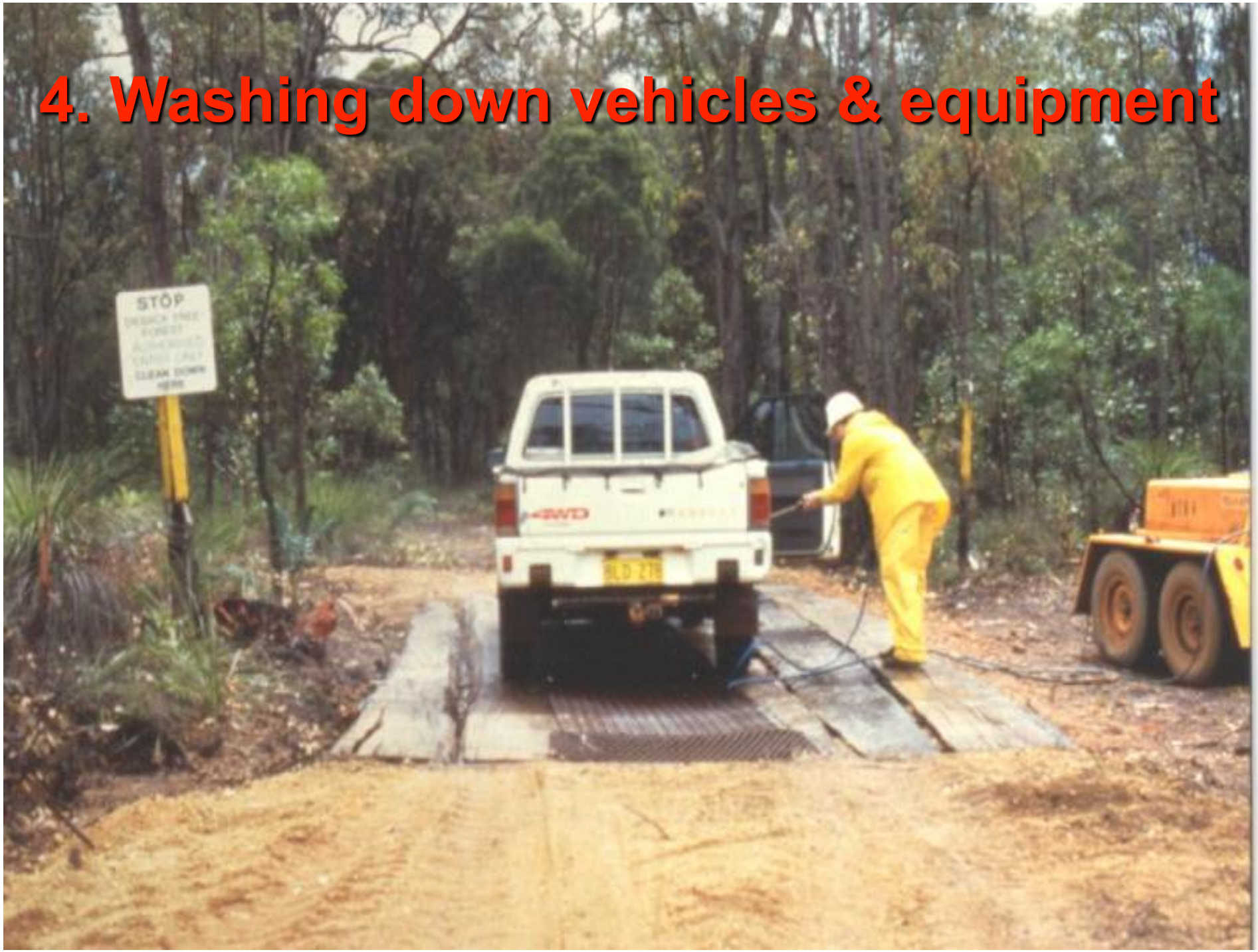


2. people movement



3. Preventing water movement

4. Washing down vehicles & equipment



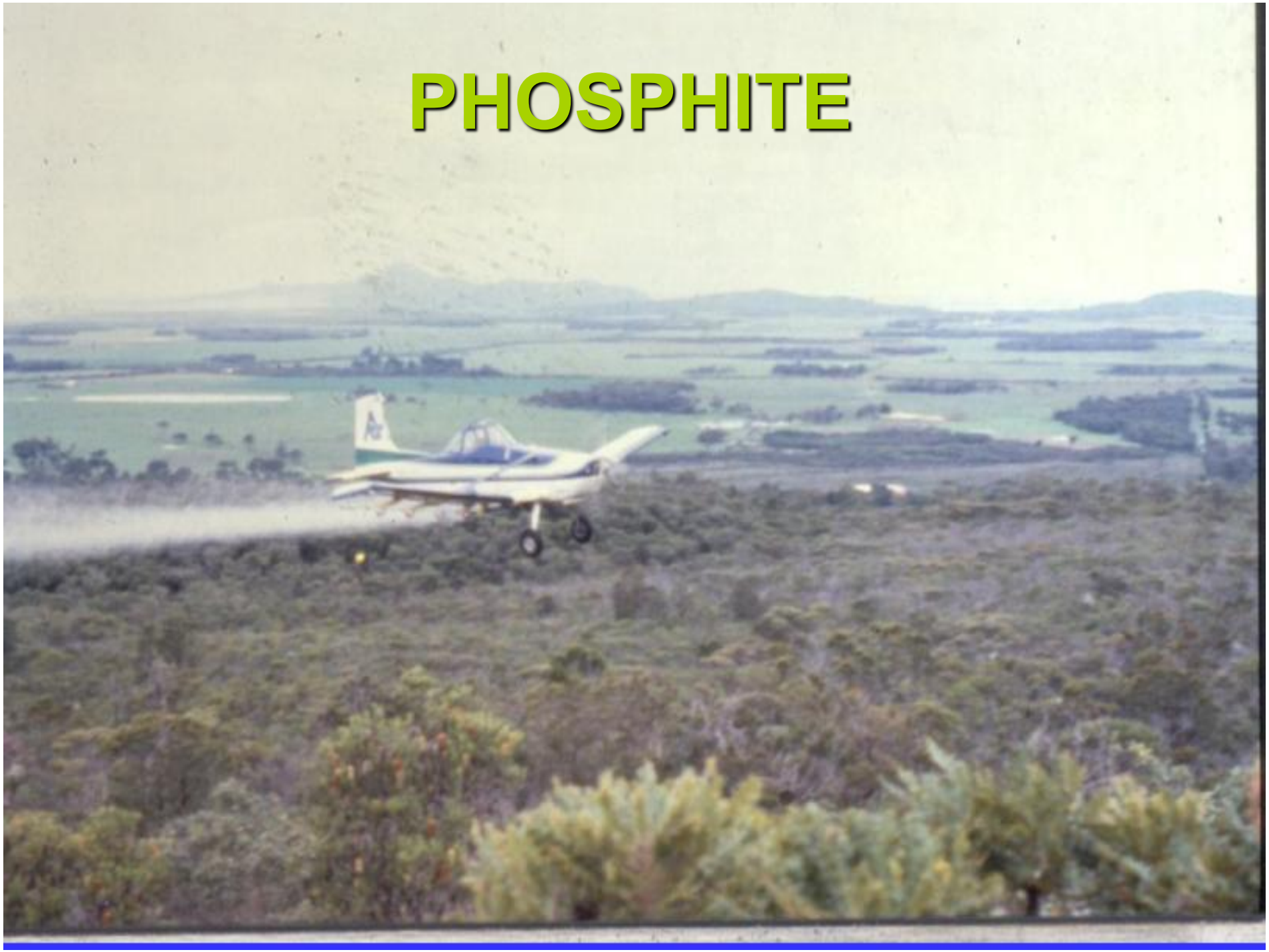
Importance of Hygiene/ Quarantine

- Isolates vary in capacity to cause disease
  - so do not want to move isolates in contaminated soil or infected plants between locations/regions/countries

Importance of Quarantine

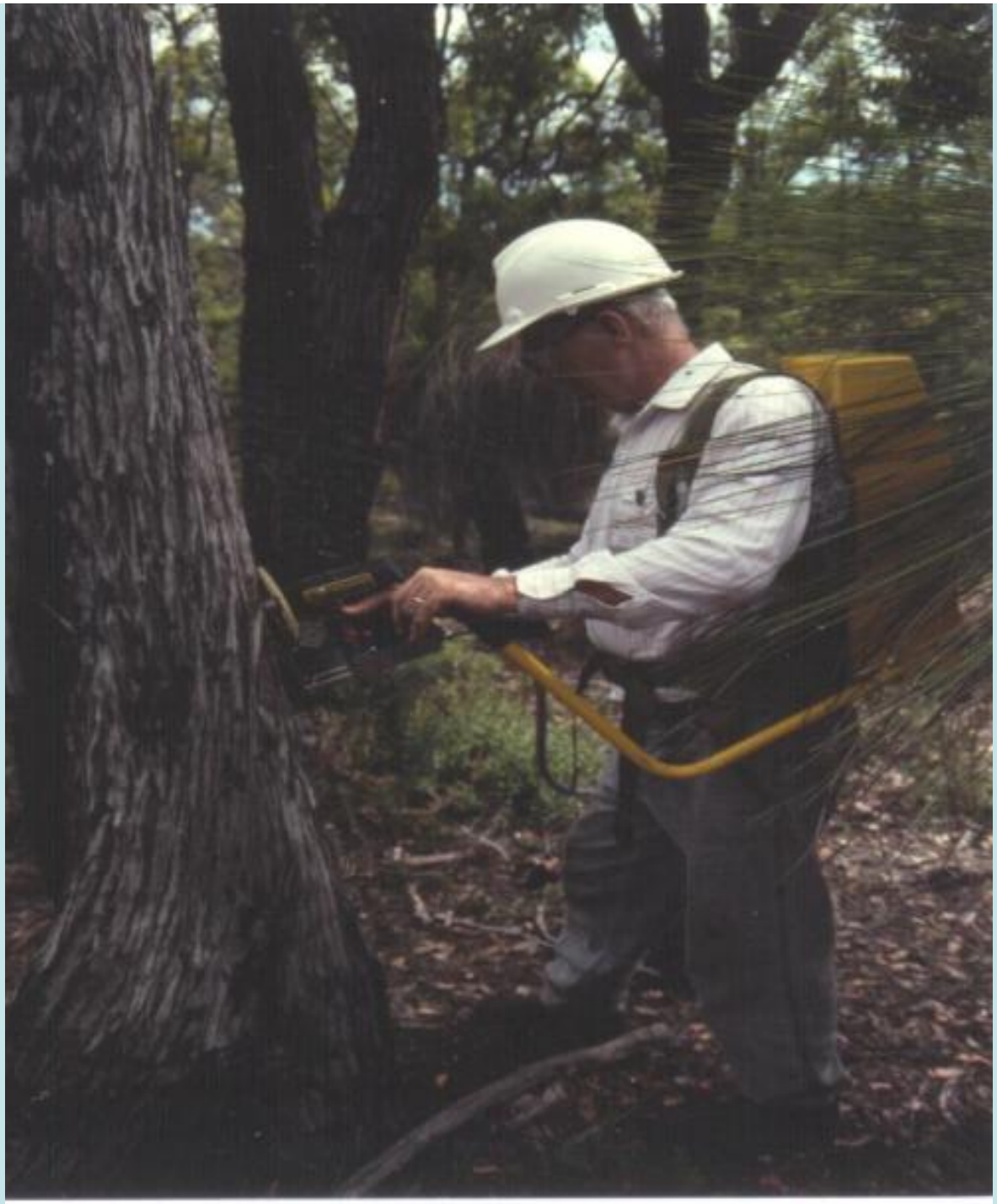
- What is disease-free material?
- False negatives using baiting and plating onto selective media
- Use of wetting and drying techniques can give recoveries in 2 of every 10 plants sampled

PHOSPHITE



Phosphite

- Unique fungicide as translocated in xylem and phloem
- Trunk injection, soil drench & foliar sprays
- Direct and indirect action *in planta*
- Controls many *Phytophthora* diseases



Effectiveness of Phosphite

- Phosphite contains, but does not stop colonization by *P. cinnamomi* in the majority of plant species (trunk injection can last for 6 years).
- It does not always stop sporulation and zoospore release from treated but infected plant material.

Potential adverse effects of phosphite

- Phytotoxicity
- Reduced plant reproductive capacity
- Production of phosphite tolerant strains of *P. cinnamomi* (?)

CONCLUSION

Disease control in natural plant communities must involve:-

- Quarantine and hygiene
- Phosphite

Oak root canker
(*Phytophthora cinnamomi*)

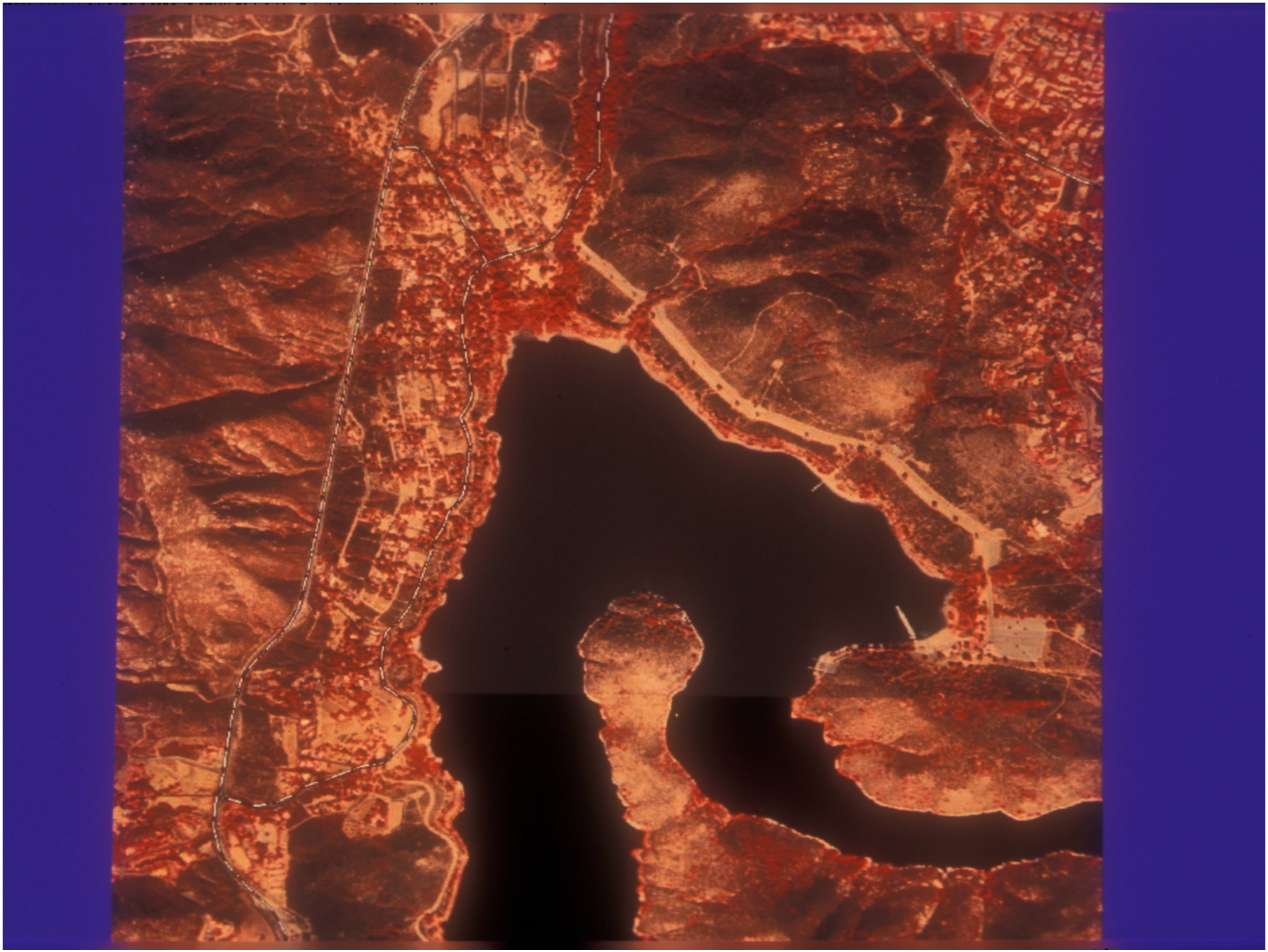
- **Species originally from PNG or Borneo, a common agricultural pathogen**
- **Soilborne, waterborne common in the wild in other parts of the US**
- **If host not extremely susceptible, predisposing factors needed for mortality to occur (e.g. oaks in Southern Europe)**
  - **Dry spell**
  - **Man-induced ecological alterations**

P. cinnamomi causes Littleleaf disease of pines on former-agricultural soils with hardpan in the Eastern US

Problem: Oak decline

Locations:

- Del Dios Area (Lake Hodges)
- County Parks
- Rural Areas



Oak Tree Survey at Del Dios

Results:

Of 474 *Quercus agrifolia* trees,
27% had bleeding cankers on the trunk.

Of 86 *Quercus engelmannii* trees,
none showed bleeding.

Pathogenicity Tests

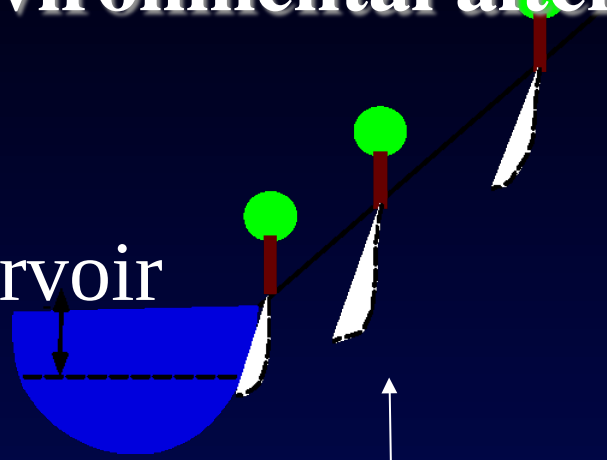
September Results:

Q. agrifolia	135 mm lesions
Q. engelmannii	49 mm lesions
Control	no lesions

Temperature: 21, 24, 18° C

Example of man-induced environmental alteration

Phytophthora cinnamomi
Introduced on
Coast Live Oak
San Diego Co.



Oaks at mid-slope experience fluctuations in the water table level: if infected by *P. cinnamomi* become extremely weak and attractive to insects

Ione manzanita: endangered species

Ione
Extremely harsh ecosystems, serpentine soild (very acidic, rich in Fe++), mining operations

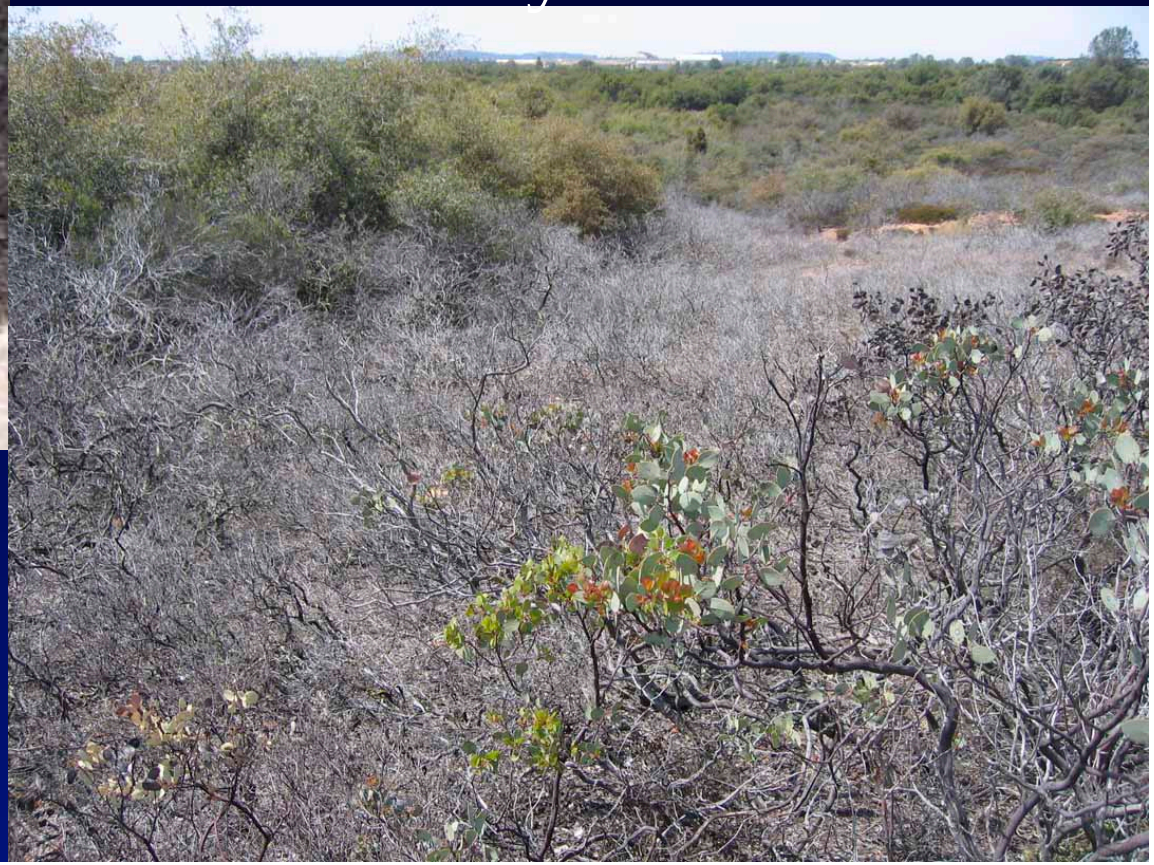
Two major components of plant cover are manzanitas:

A. viscida (white manzanita)
A. myrtifolia (ione manzanita)

Ione manzanita is a rare endemic species of the Ione area, one that has well adapted to the local conditions, but it is currently in the list of US threatened species

Because of almost total susceptibility to soilborne *P. cinnamomi*

Genetic diversity of Pc in Ione is staggering, it includes all of the diversity present in California natural ecosystems




PLANT DISEASE AREA
THE VEGETATION BEHIND THIS SIGN MAY CONTAIN
A PLANT DISEASE THAT IS EASILY SPREAD
THIS AREA CLOSED
TO ALL PUBLIC USE
FOR FURTHER INFORMATION CALL BLM: (916) 985-4474

How can we explain this diversity?

- At least three introductions of three distinct strains
- Populations large enough that additional diversity generated locally (soil environment favorable to pathogen)
- One dominant strain is also present in Ca Christmas tree farms also matching a strain from a severe outbreak of oak mortality in Colima. This strain is novel

A Microsatellite Analysis Used to Identify Global Pathways of Movement of *Phytophthora cinnamomi* and the Likely Sources of Wildland Infestations in California and Mexico

María Socorro Serrano,[1,2] Todd Osmundson,[1,3] Alejandra Almaraz-Sánchez,[1,4] Peter J. P. Croucher,[1] Tedmund Swiecki,[5] Dionicio Alvarado-Rosales,[4] and Matteo Garbelotto[1,†]

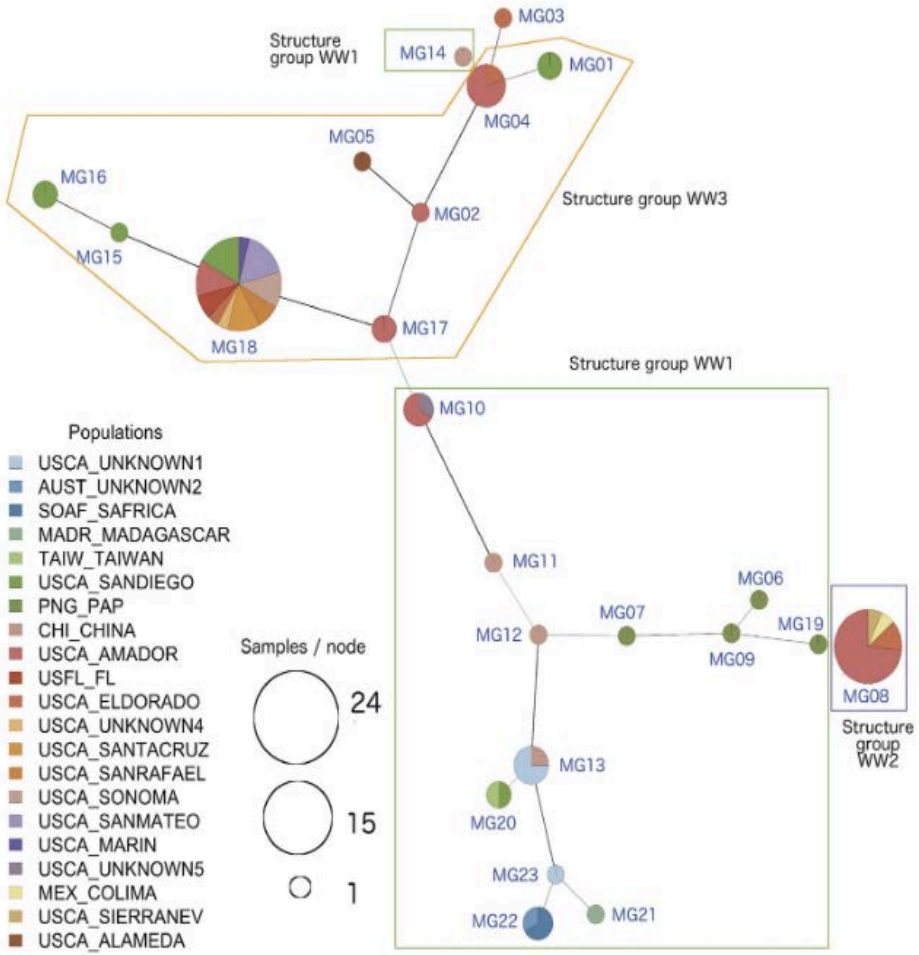
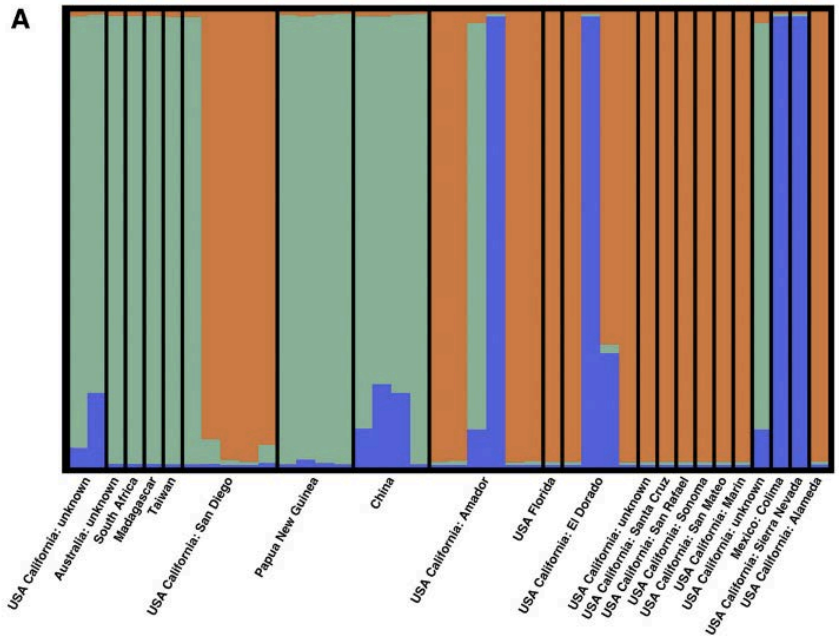
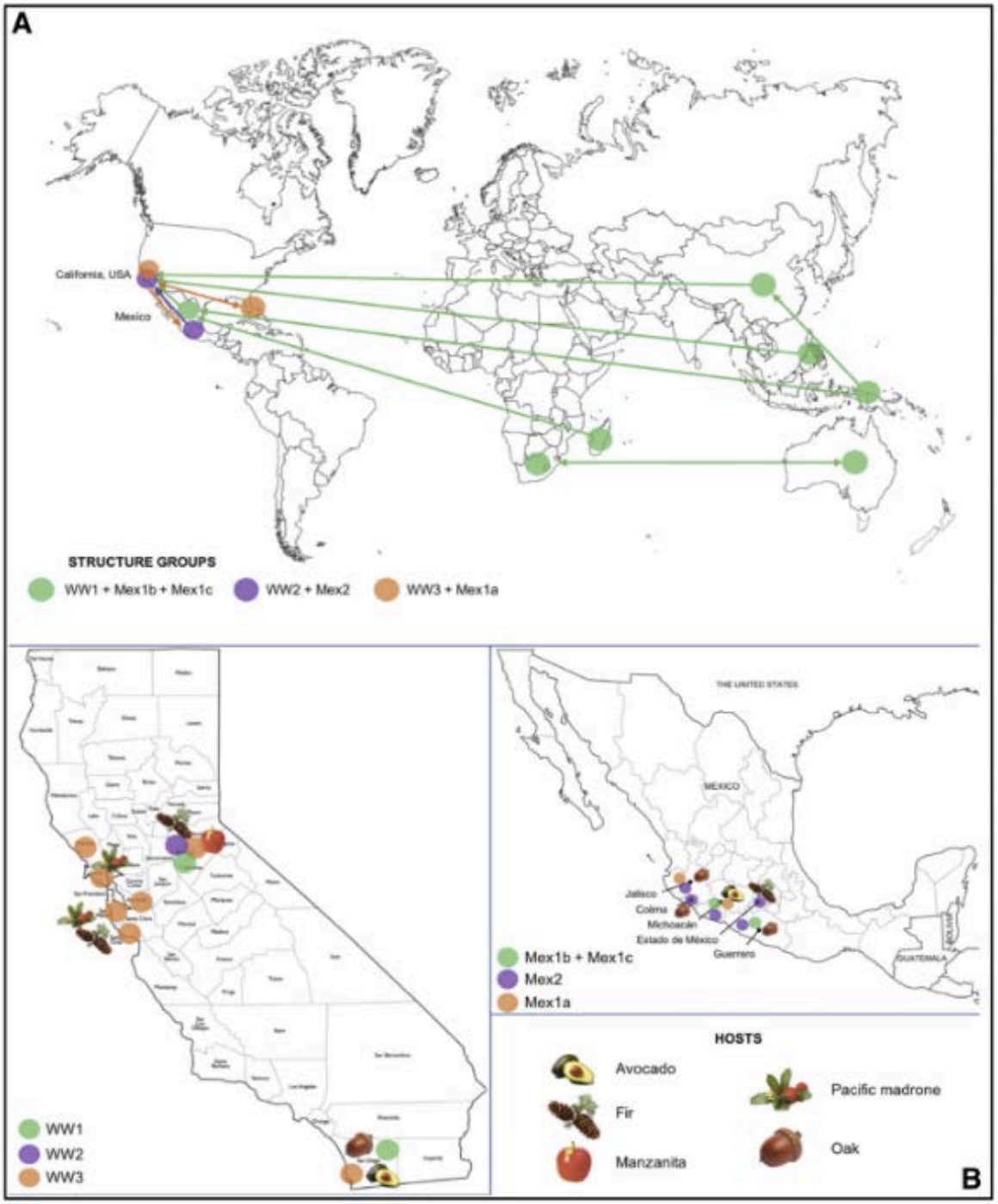


Fig. 3. Minimum spanning network based on the matrix of pairwise Bruvo genetic distances visualizing relatedness among multilocus genotypes (MGs) in the worldwide/California analysis.



3 genetically distinct groups
WW1 Native and first world spread
WW3-North America a few hundred years
WW2- Recent, derived from WW1 introduced From Mexico


A
California, USA
Mexico
STRUCTURE GROUPS
WW1 + Mex1b + Mex1c
WW2 + Mex2
WW3 + Mex1a
THE UNITED STATES
MEXICO
GUATEMALA
Jalisco
Colima
Michoacán
Estado de México
Guerrero
Mex1b + Mex1c
Mex2
Mex1a
WW1
WW2
WW3
HOSTS
Avocado
Fir
Manzanita
Pacific madrone
Oak
B

And in the greater SF Bay Area

- *P. cinnamomi* associated with root infections and tree decline of California Bay laurels and Pacific madrones
- Normally in association with human disturbance (roads, landscaping, urban development etc) including estates with lush gardens
- Interesting genetic homogeneity of strains.

Where does *P. cinnamomi* come from?

- Avocado orchards once surrounded oak woodlands infected by *P. cinnamomi*
- Christmas tree farms are above the Ione manzanita range

Microsatellite analysis (5 loci) differentiated 22 clones representative of common lineages around the world

- In San Diego Co., one clone only was found. It matched one of the three clones found on avocado worldwide. It also matched one isolate from an avocado orchard in SD county
- Surprisingly, three/four clones found in manzanita (one common on ornamental plants, one in orchards and one matching an outbreak on oaks in Mexico). Two of the clones were also found on Christmas trees

Hidden costs of activities on natural ecosystems

Once introduced, these organisms are almost impossible to eradicate

Phytophthora lateralis as the cause of extensive mortality of Port Orford cedar in the Pacific Northwest

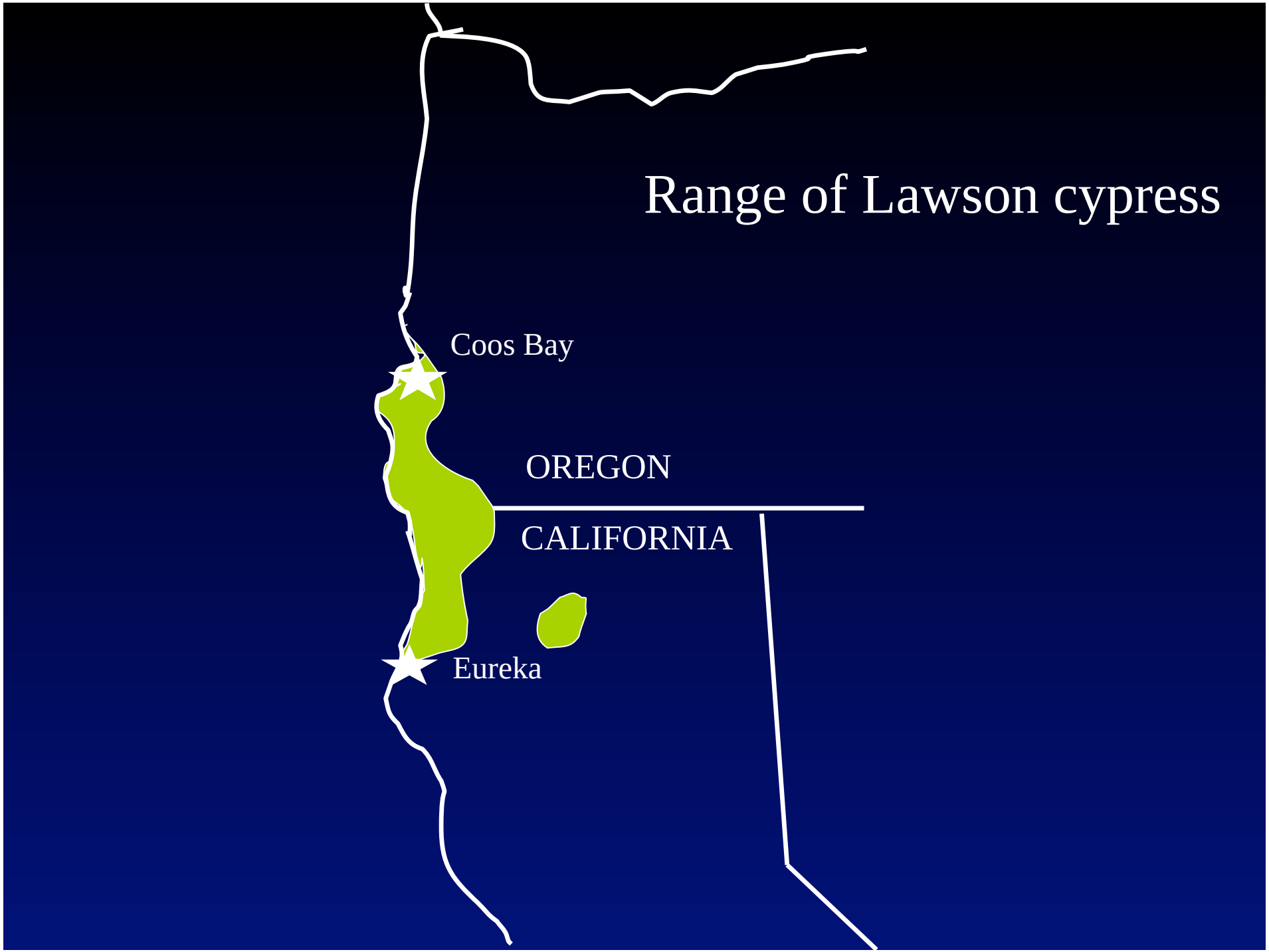

Range of Lawson cypress
Coos Bay
OREGON
CALIFORNIA
Eureka

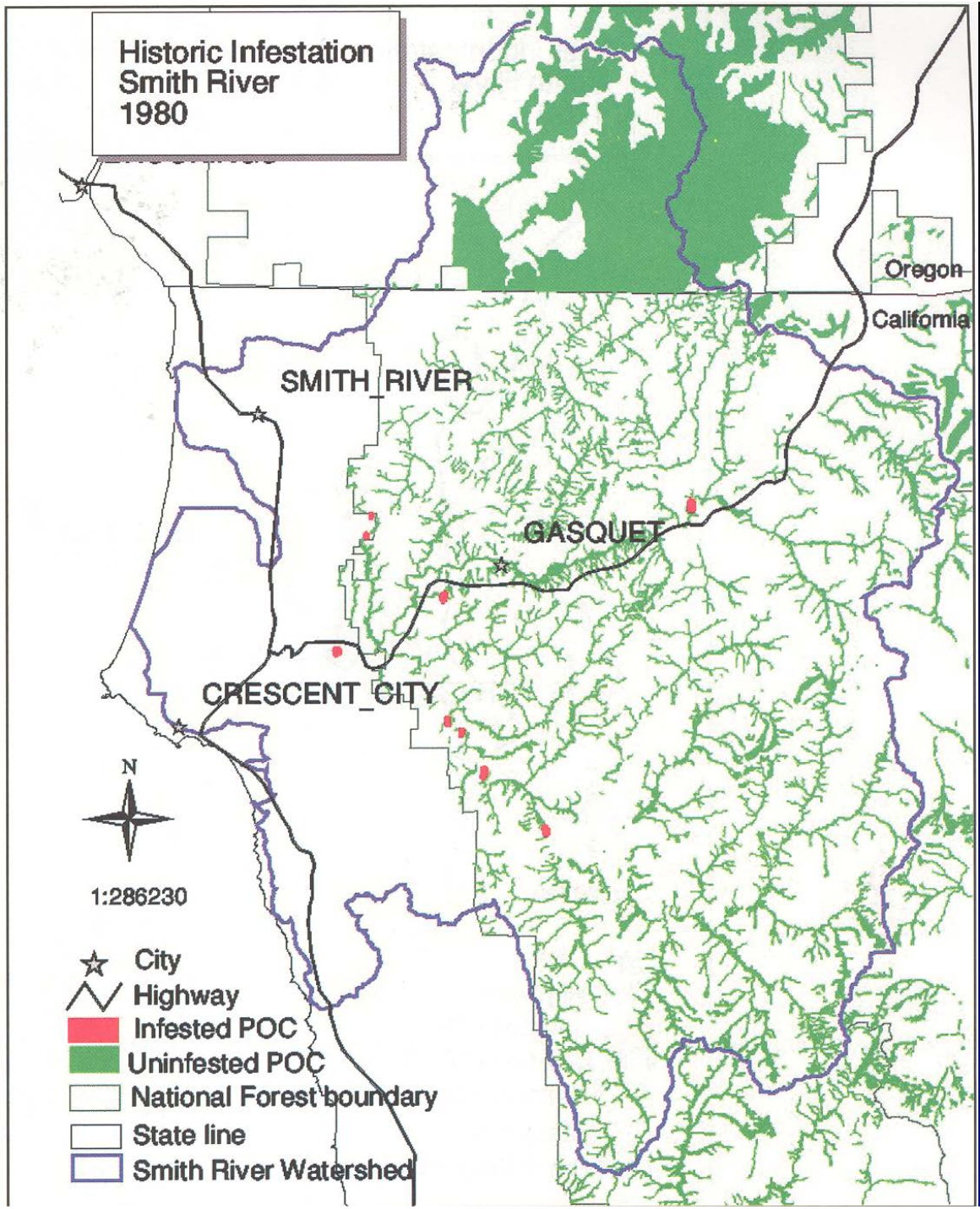

Photo: Paul Reeser

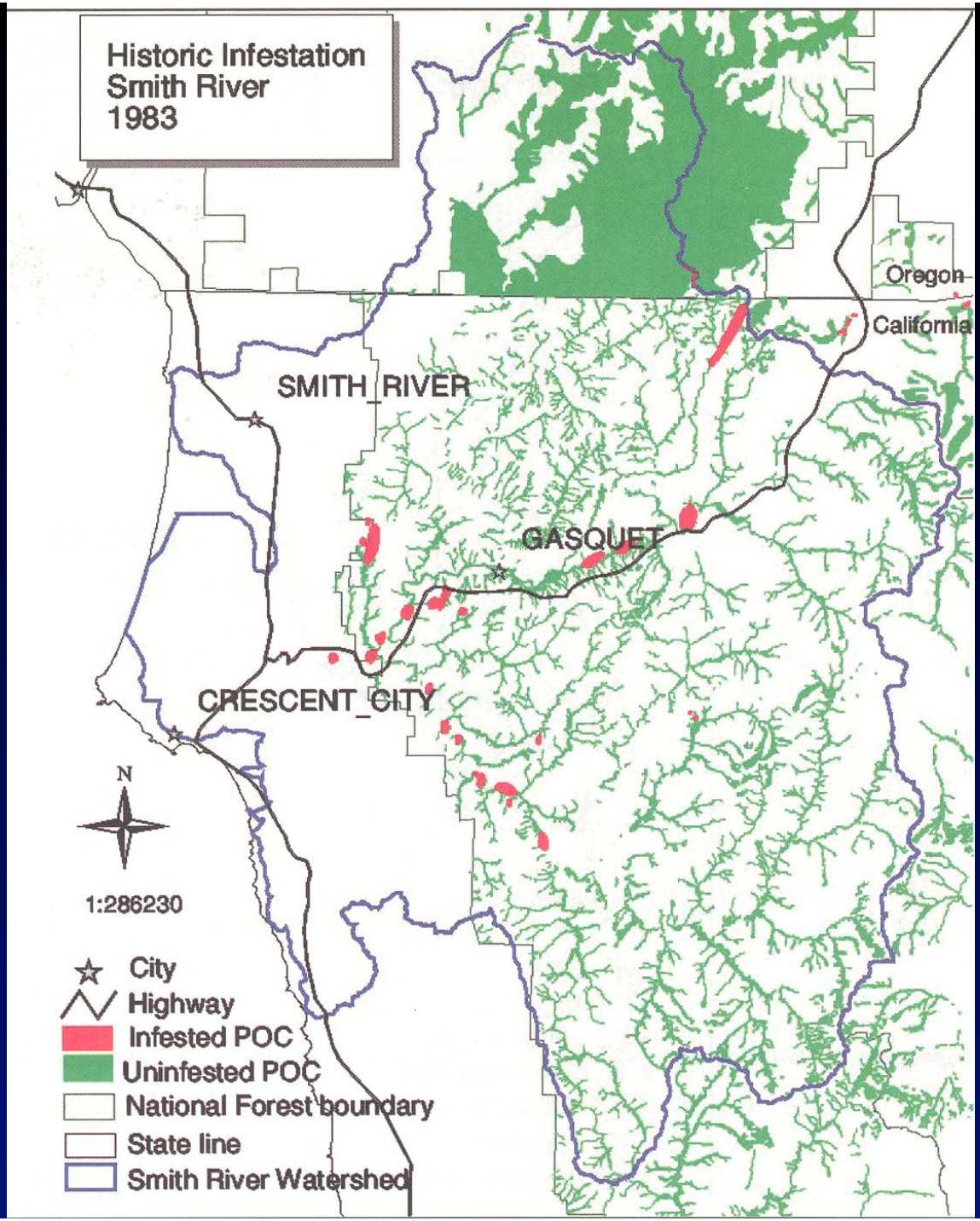
Port Orford cedar root disease

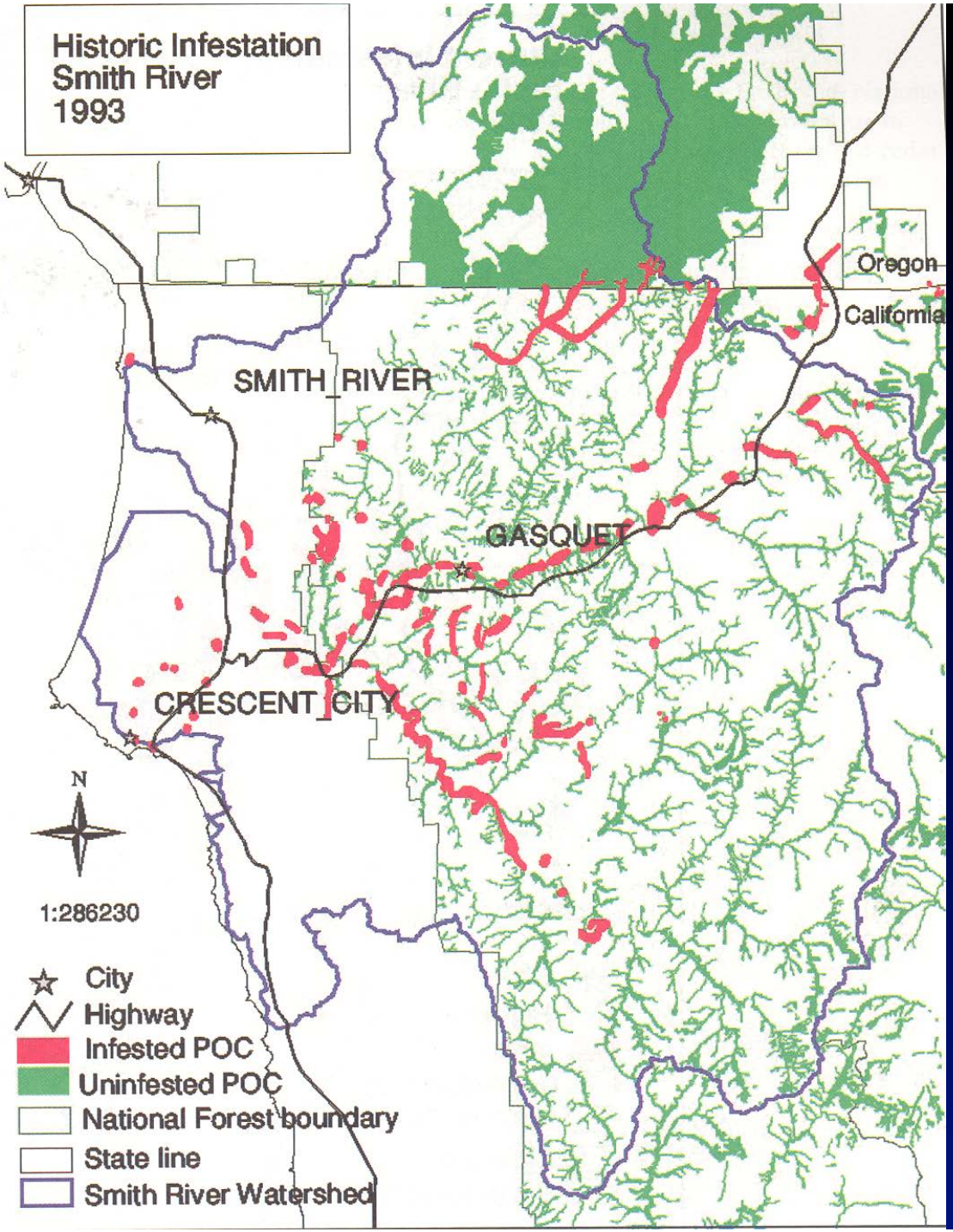
(*Phytophthora lateralis*)

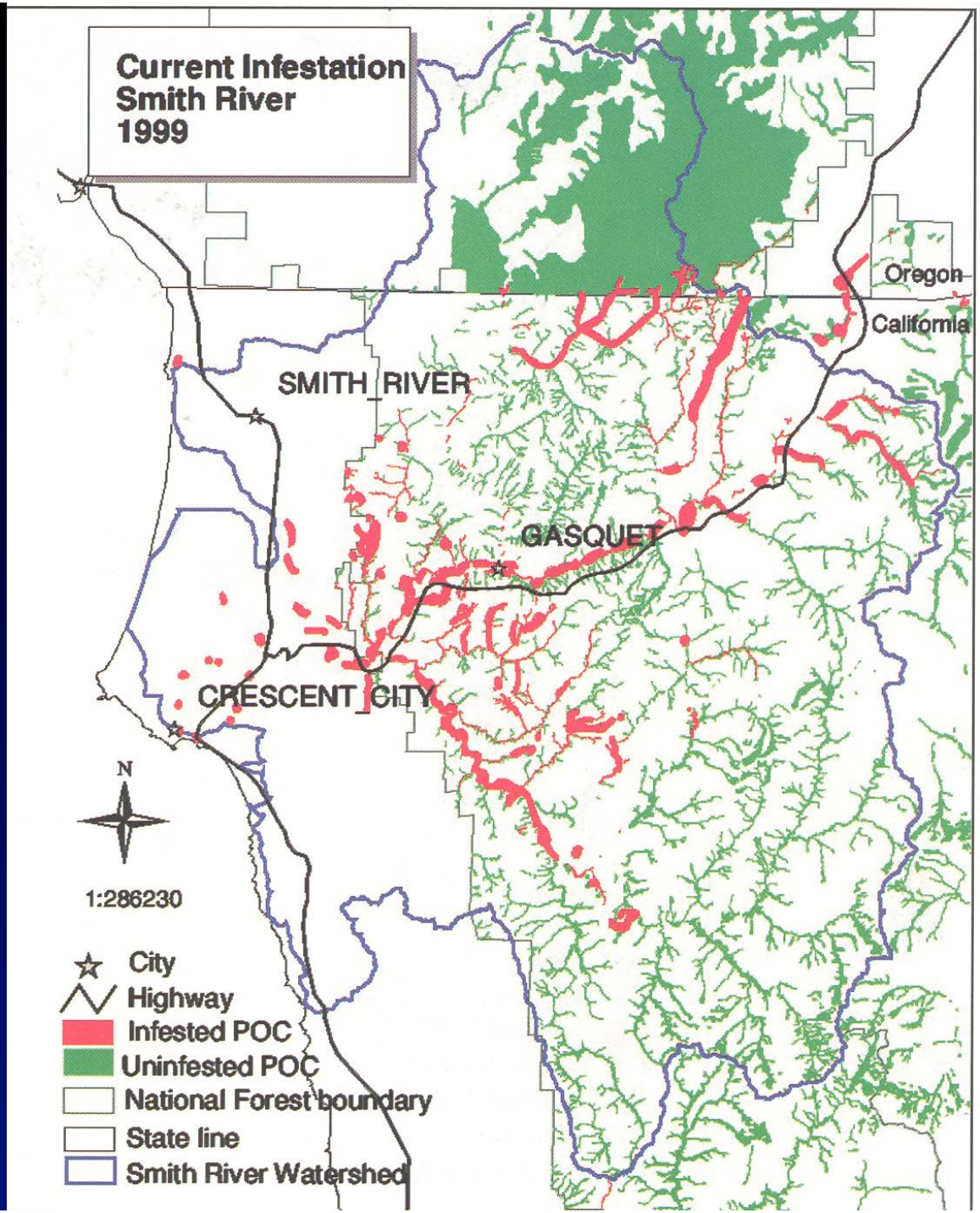
- **Introduced in California from Oregon, to Oregon from Europe?**
- **Originally confined to plant nurseries**
- **Spread by soil and water movement**
- **Prevention by isolation possible**
- **Genetic resistance program**

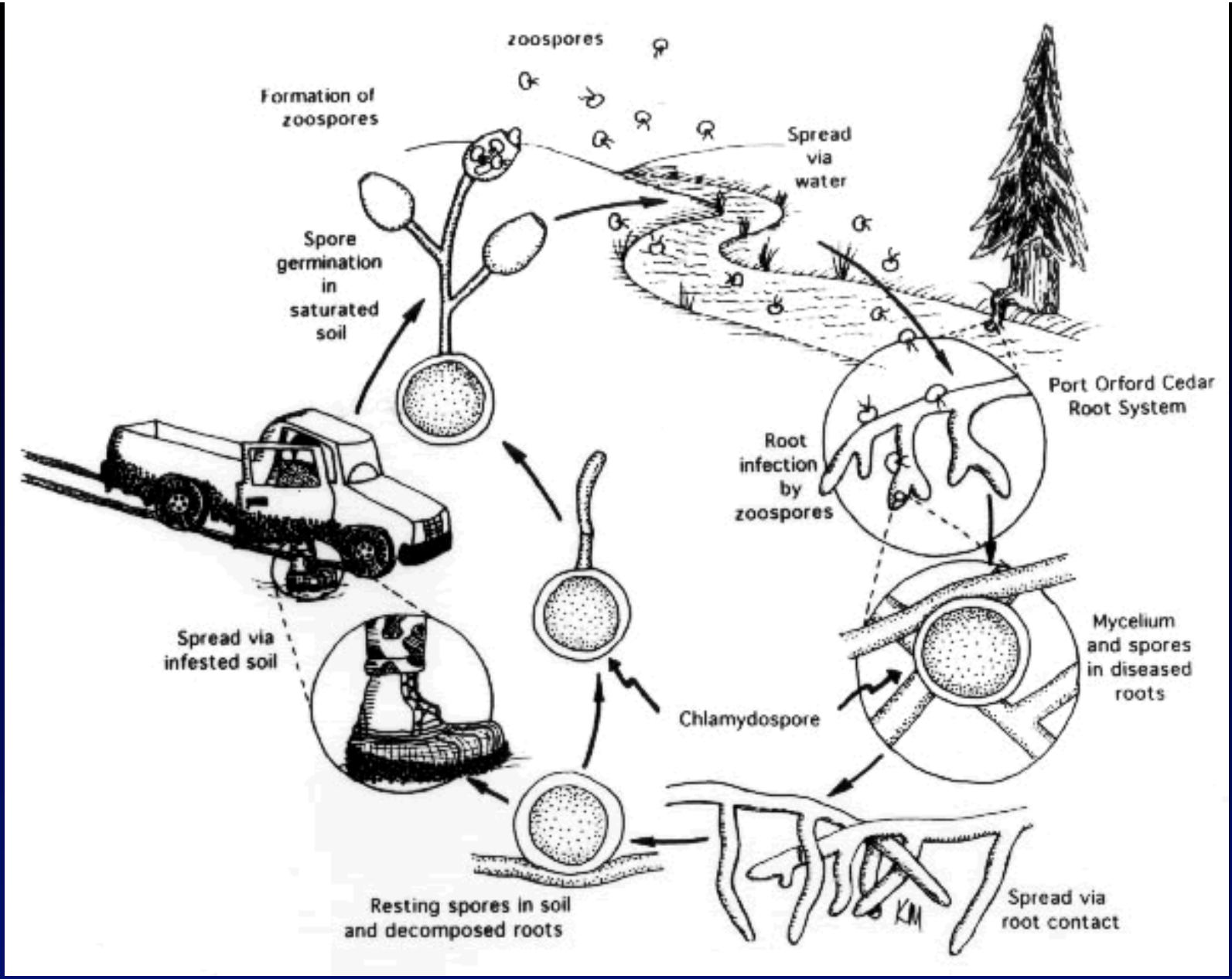
Association of mortality with roads and streams


Historic Infestation
Smith River
1980
Oregon
California
SMITH_RIVER
GASQUET
CRESCENT_CITY
N
1:286230
City
Highway
Infested POC
Uninfested POC
National Forest boundary
State line
Smith River Watershed


Historic Infestation
Smith River
1983
Oregon
California
SMITH_RIVER
GASQUET
CRESCENT_CITY
N
1:286230
City
Highway
Infested POC
Uninfested POC
National Forest boundary
State line
Smith River Watershed


Historic Infestation
Smith River
1993
Oregon
California
SMITH RIVER
GASQUET
CRESCENT CITY
N
1:286230
City
Highway
Infested POC
Uninfested POC
National Forest boundary
State line
Smith River Watershed


Current Infestation
Smith River
1999
Oregon
California
SMITH RIVER
GASQUET
CRESCENT CITY
N
1:286230
City
Highway
Infested POC
Uninfested POC
National Forest boundary
State line
Smith River Watershed


zoospores
Formation of zoospores
Spread via water
Spore germination in saturated soil
Port Orford Cedar Root System
Root infection by zoospores
Mycelium and spores in diseased roots
Chlamydospore
Spread via infested soil
Resting spores in soil and decomposed roots
Spread via root contact

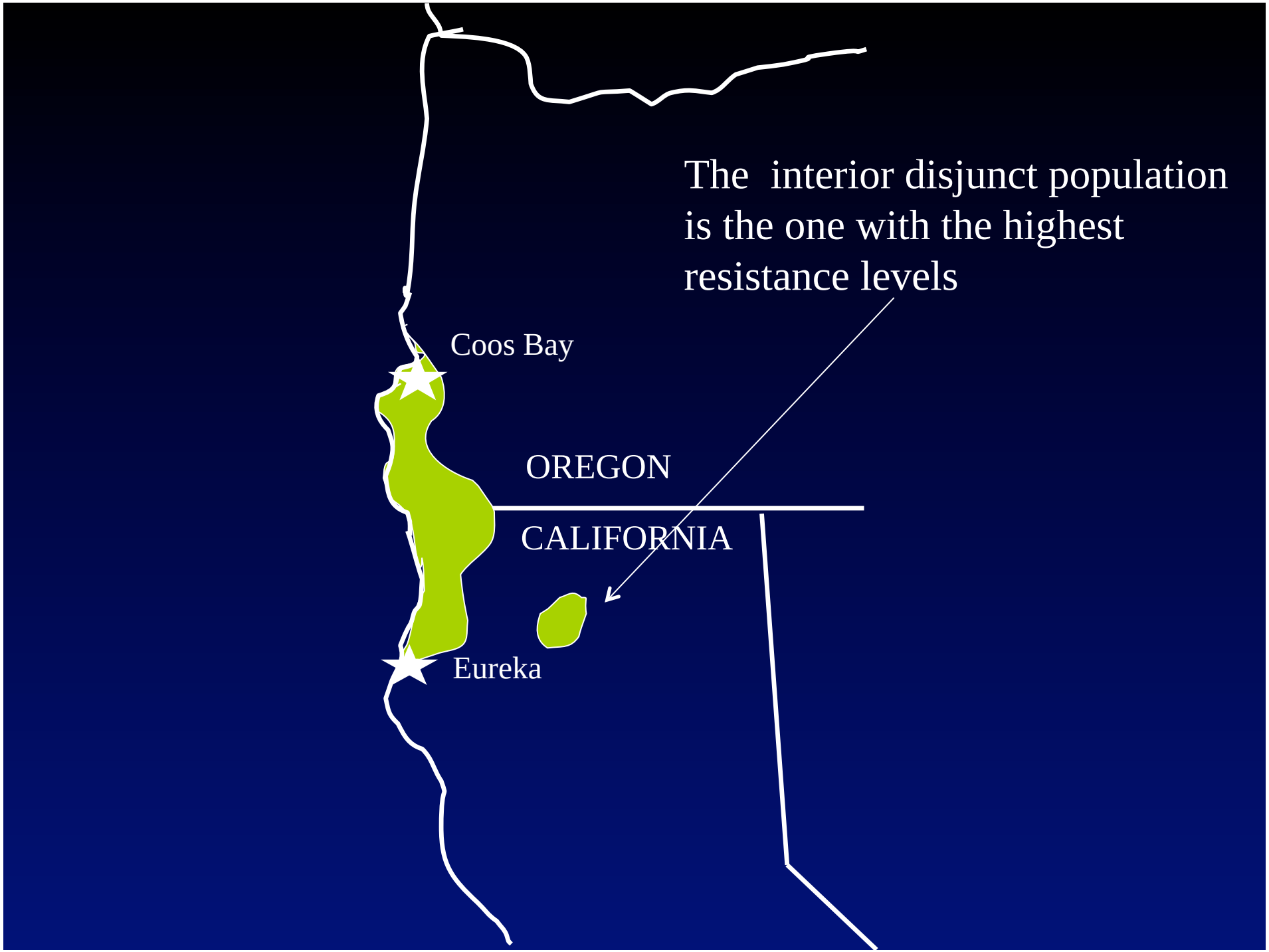
Genetic Resistance Programs

- Identify distinct populations, how far will I need to go to sample populations that have not shared alleles recently?
- Identify permanent signatures in populations (maternal inheritance)
- Quantify amount of diversity present in populations, to assist with required sampling intensity
- Collect seed from mother trees (half sibs) or artificially pollinate mother trees to analyze full sibs
- Germinate trees in same location to create a common garden experiment, in which the effects of different environmental conditions may be minimized

Genetic Resistance Programs (2)

- In infested areas:

- Determine if survivors have a higher average resistance to disease, this would indicate resistance is actually playing a role
- See if any genetic or phenotypic traits may be associated with increased resistance (e.g. in survivors vs entire population)
- In areas with high infestation levels, survivors may be trees with natural resistance
- Determine if natural resistance is qualitative (one gene determines presence of resistance) or quantitative (resistance is the result of the additive effects of multiple genes)
- Qualitative resistance is easier to obtain (50% of progeny will have it) but can also be breached more easily by pathogen


The interior disjunct population is the one with the highest resistance levels
Coos Bay
OREGON
CALIFORNIA
Eureka

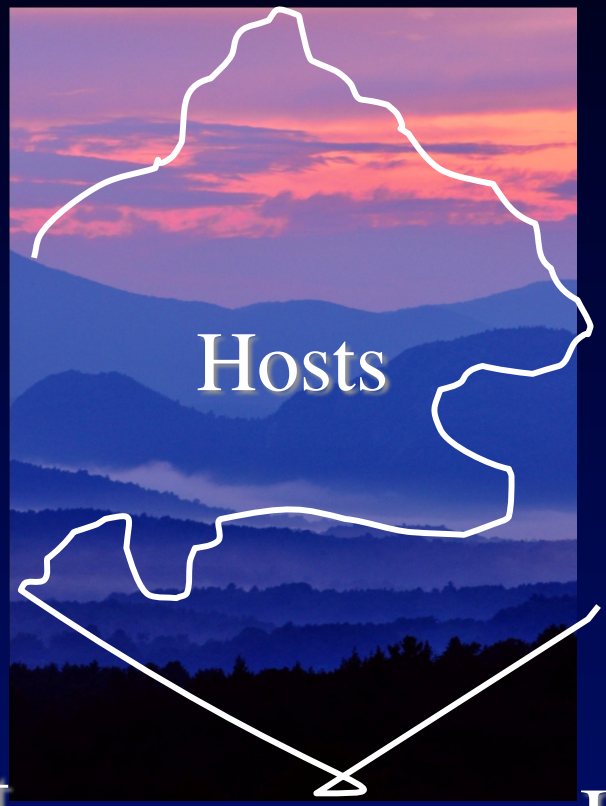
Where does it originate from?

- Recently, *P. lateralis* isolated in Taiwan where local cedars display considerable resistance
- Although a clear adaptation of *P. lateralis* to be soil- and water-borne has been shown in the Pacific Northwest, there are recent reports of aerial infections of Port Orford Cedars from Europe, suggesting a considerable plasticity

Aerial infection symptoms caused by *P. lateralis* in Europe

Sudden Oak Death: biology and management

Pathogen



Man

Environment

Interactions