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

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

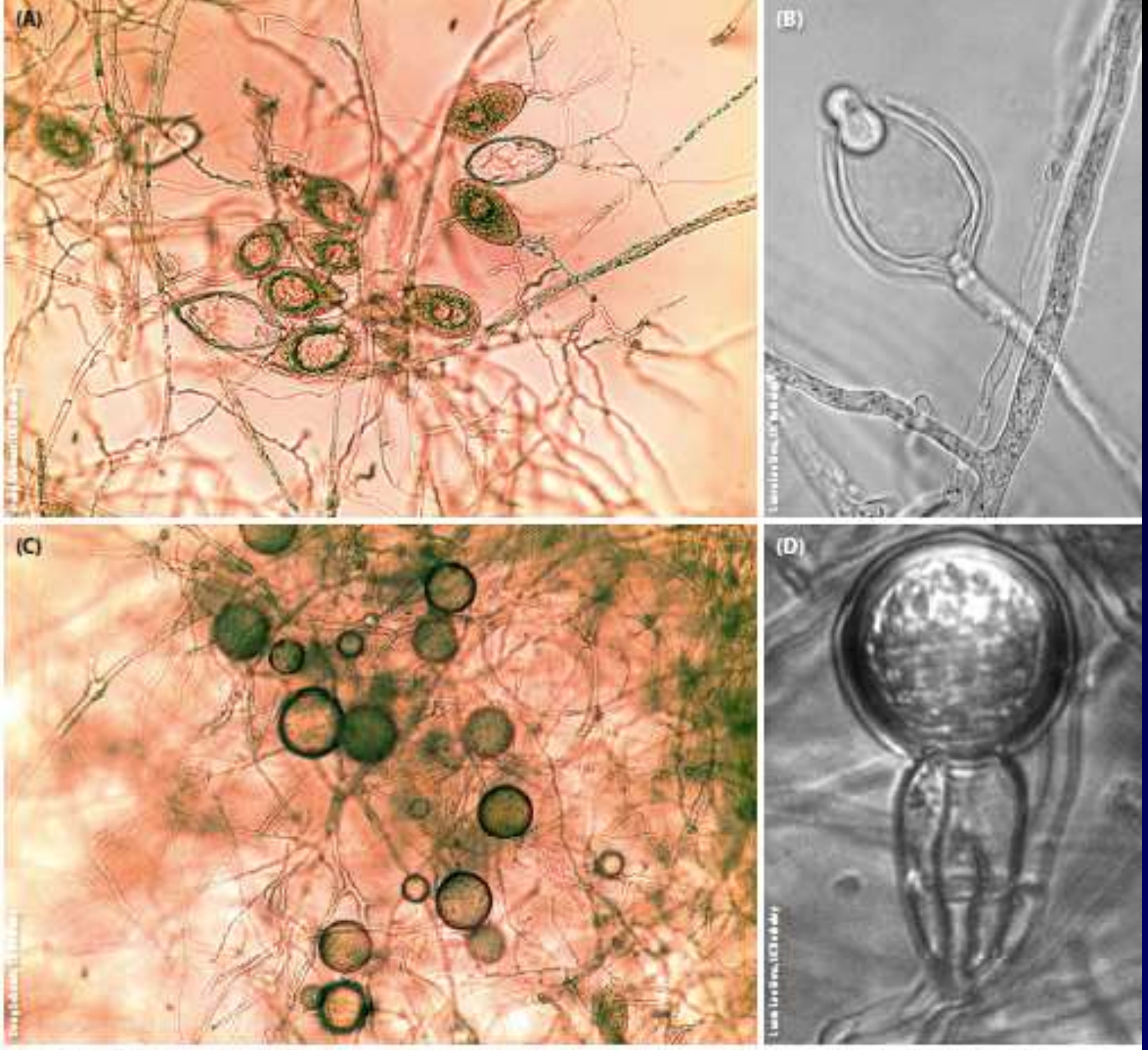


FIG. 1. Micrographs (300x magnification) of (A) sporangia of *Phytophthora ramorum*, (B) a zoospore exiting a sporangium of *Phytophthora taxon oaksoil*, (C) chlamydospores of *Phytophthora ramorum*, and (D) an oospore of *Phytophthora alni subspecies uniformis*.

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.

Phytophthora: "plant destructor"

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

100+ 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

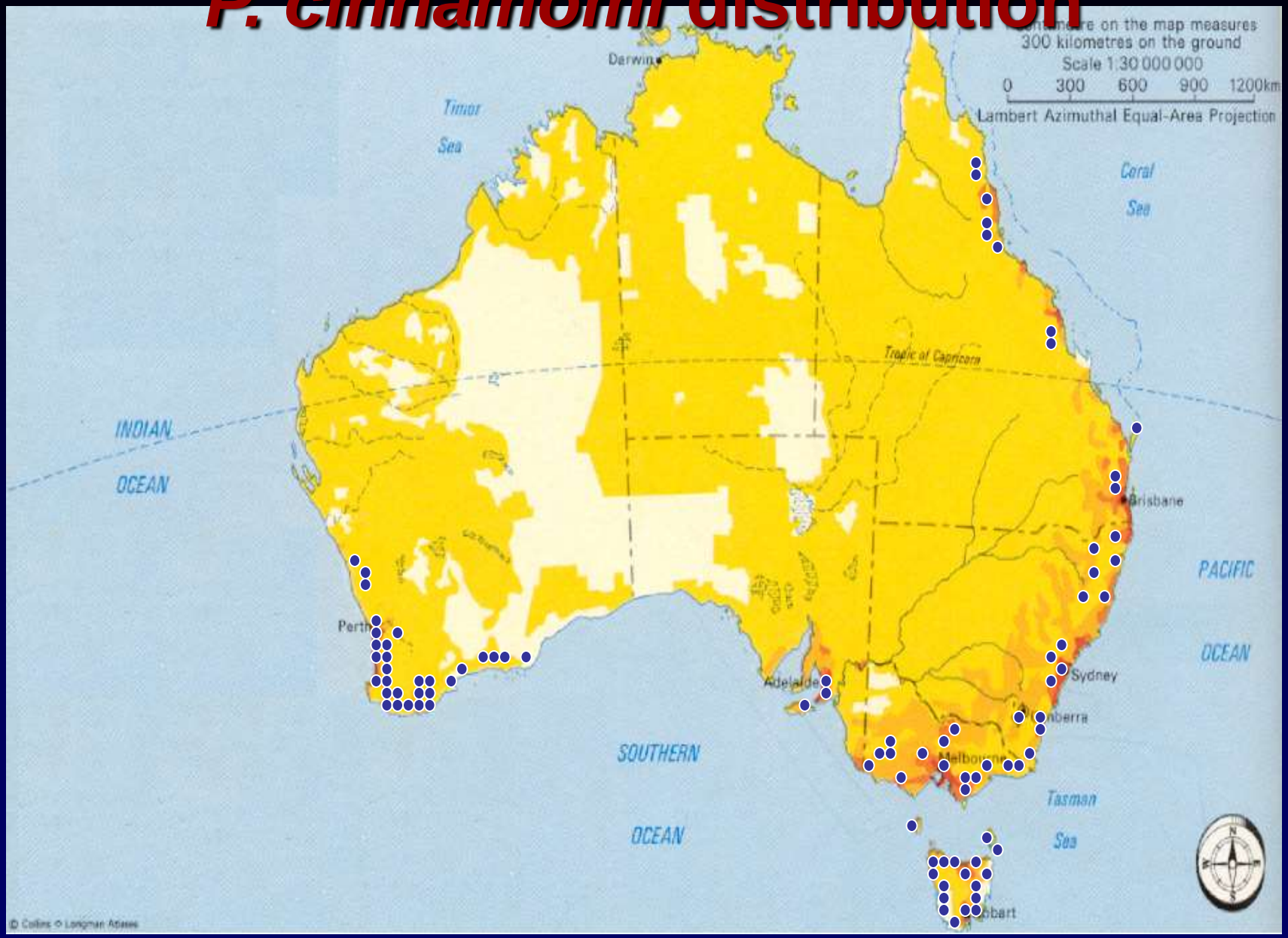
P. cinnamomi in Western Australia

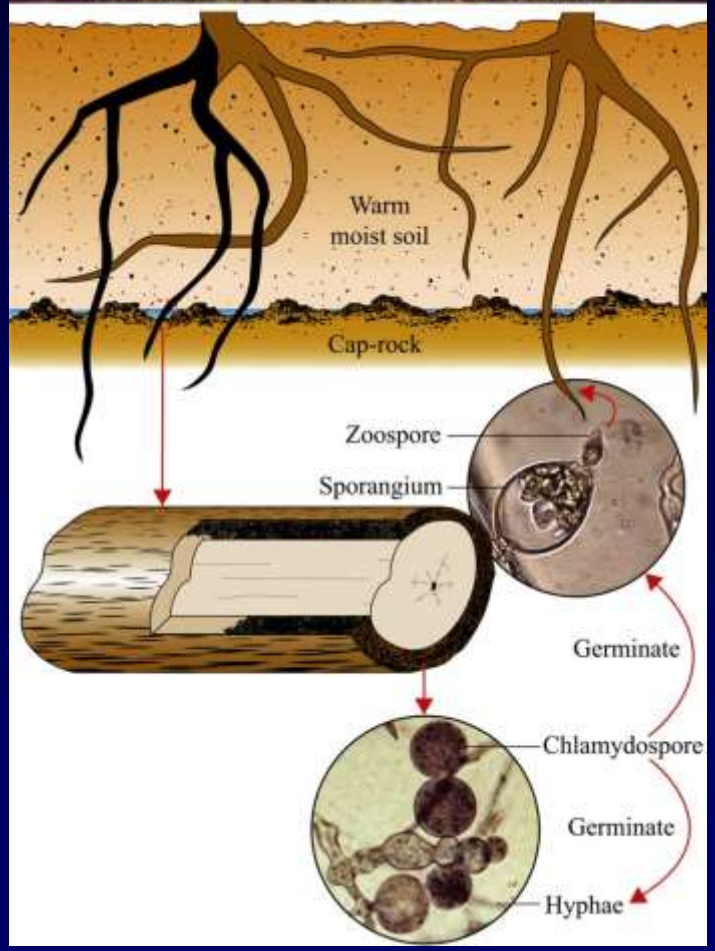
- Causes disease "jarrah dieback"
- 1921 first jarrah deaths; 1964 deaths shown to be caused by *P. cinnamomi*.
- Predominantly A2 mating type
- Three clonal lineages
- No sexual reproduction occurs

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

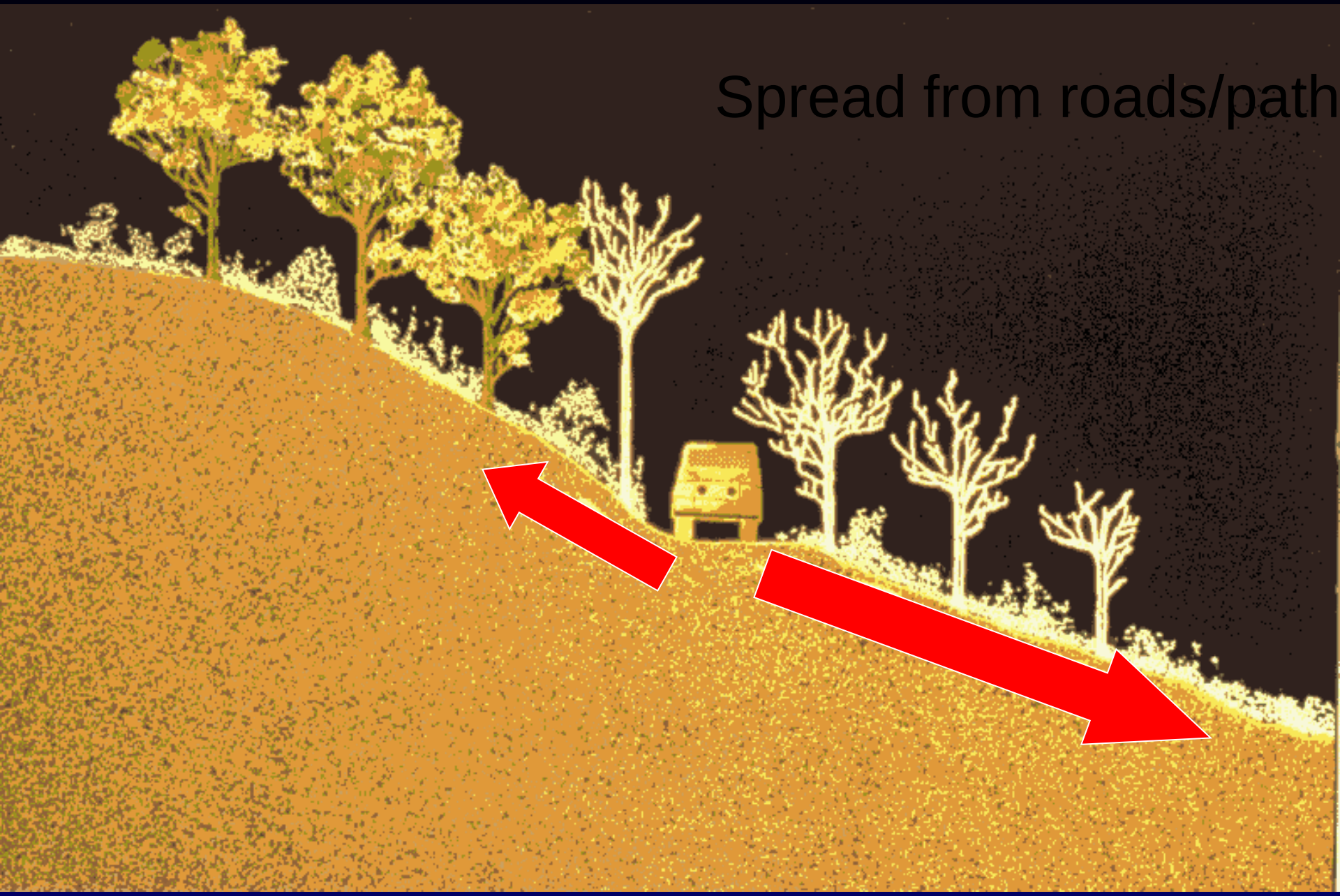
P. cinnamomi distribution



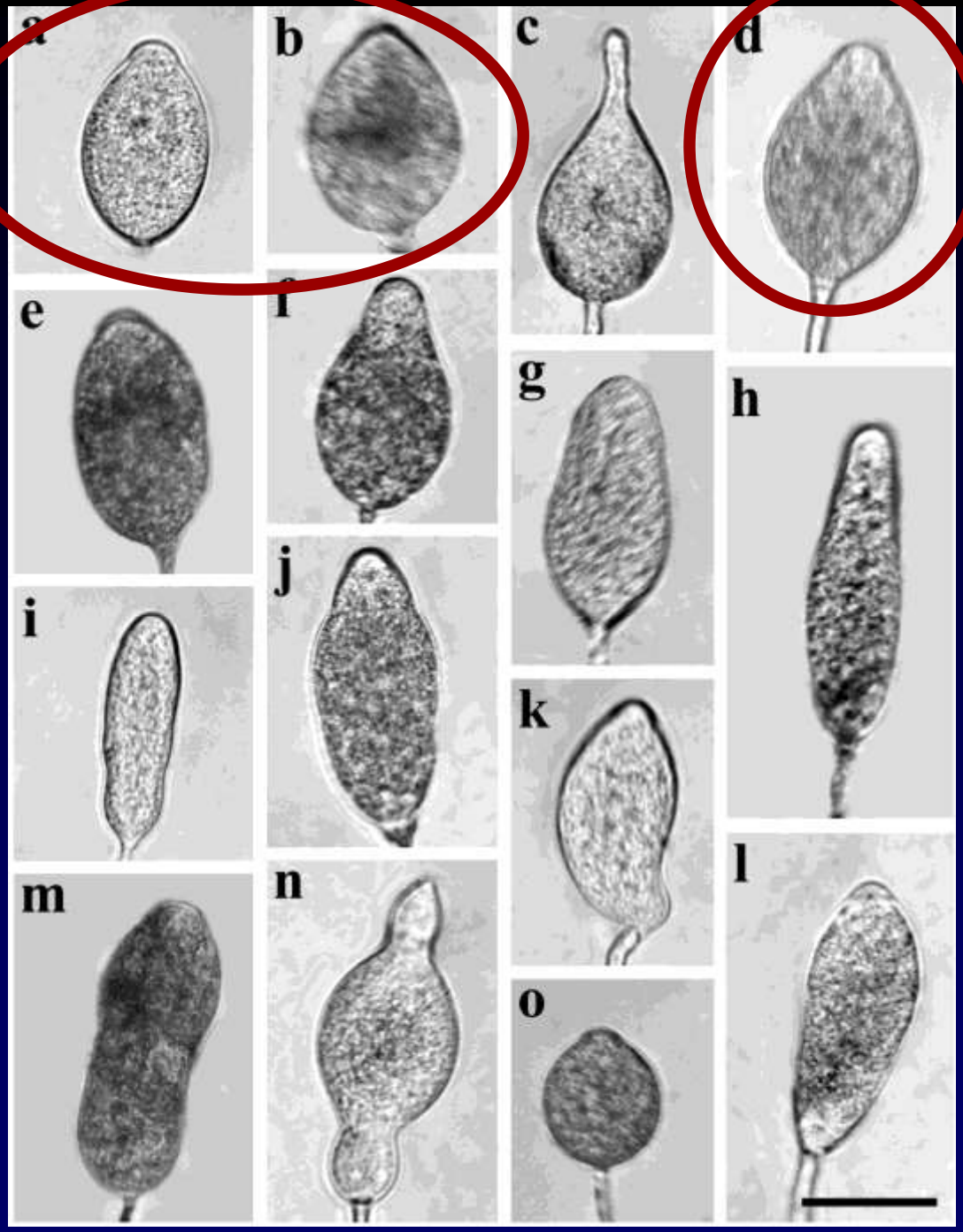

Warm
moist soil
Cap-rock
Zoospore
Sporangium
Germinate
Chlamydospore
Germinate
Hyphae

How is it spread?

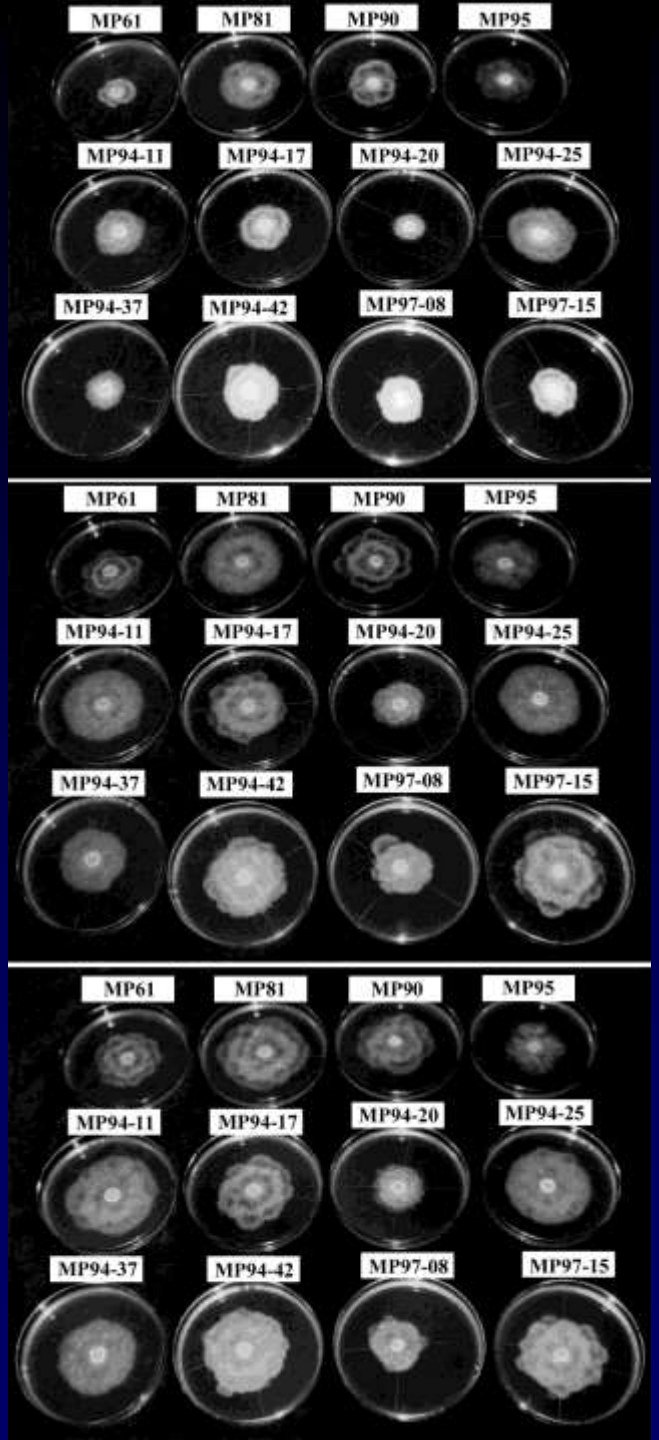
- Natural – root contact, free draining water (warm & moist; spring, summer & early autumn)
- Artificial – transport of infested soil (tyres, road making)
  - hikers (boots, tent pegs & toilet trowels)
  - planting infected nursery stock


Spread from roads/path

Typical
P. cinnamomi
sporangia



Variation in growth rate & colony morphology at different temperatures

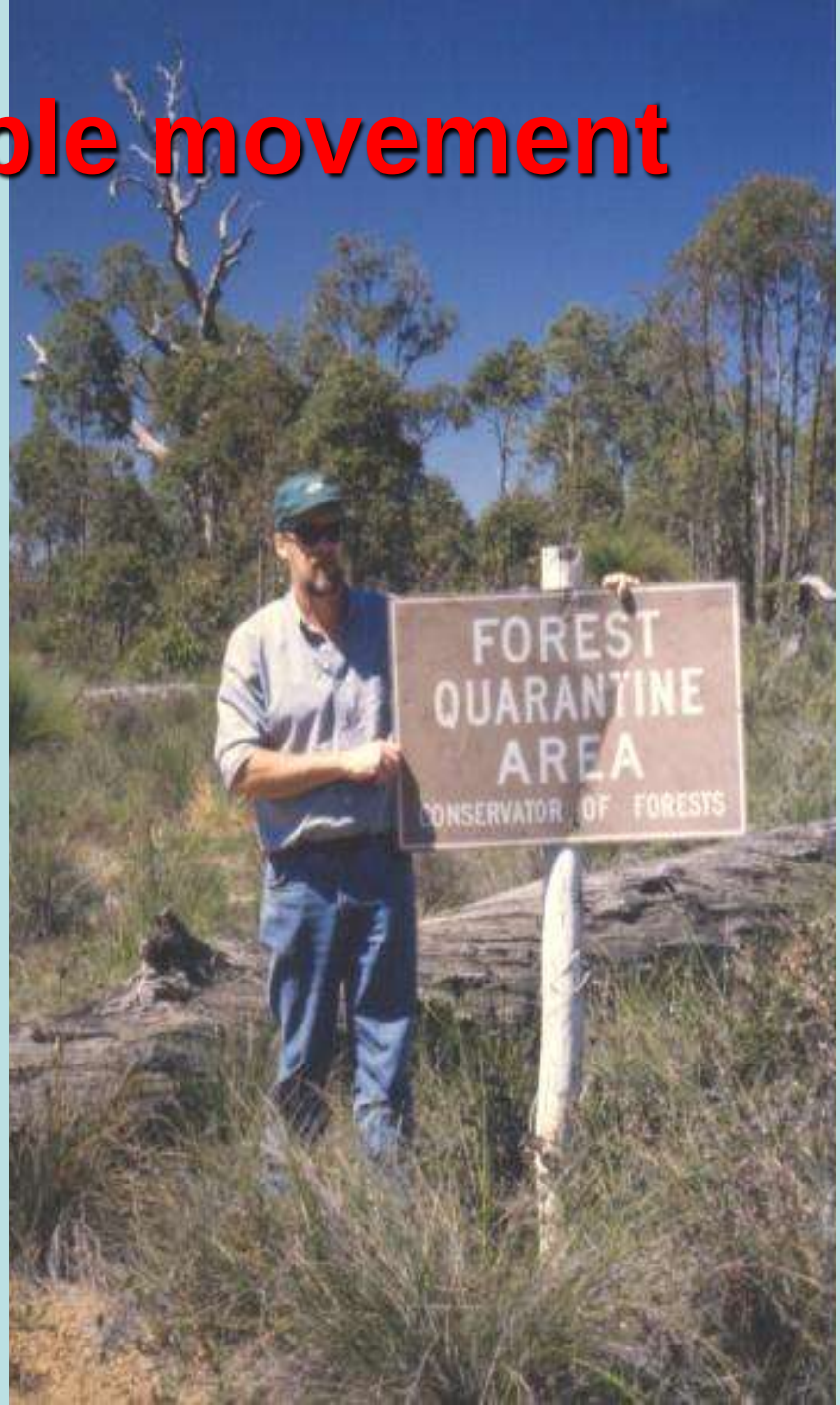


20° C

24° C

28° C

2. people movement



3. Preventing water movement

4. Washing down vehicles & equipment

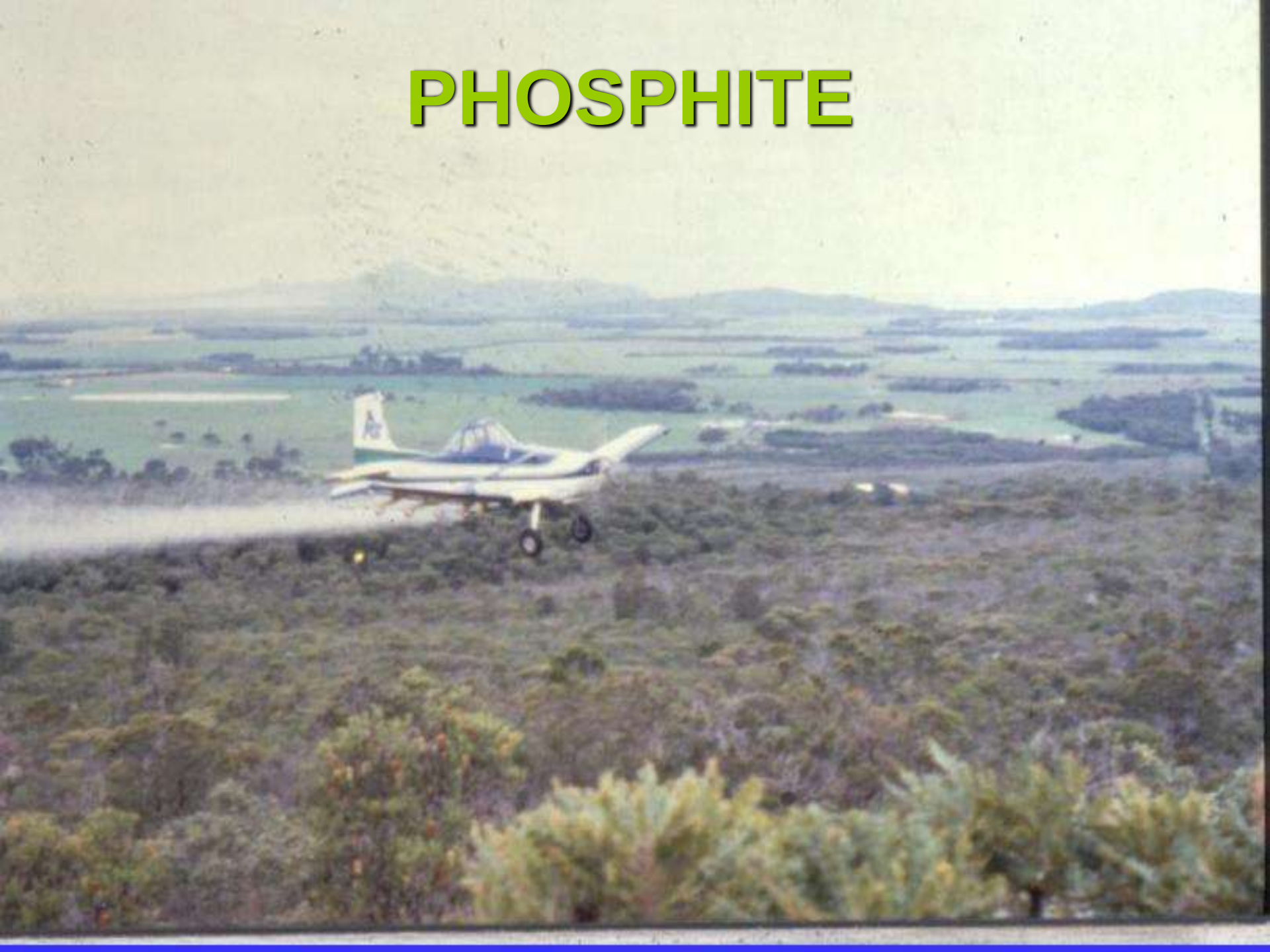
Importance of Hygiene/ Quarantine

- 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

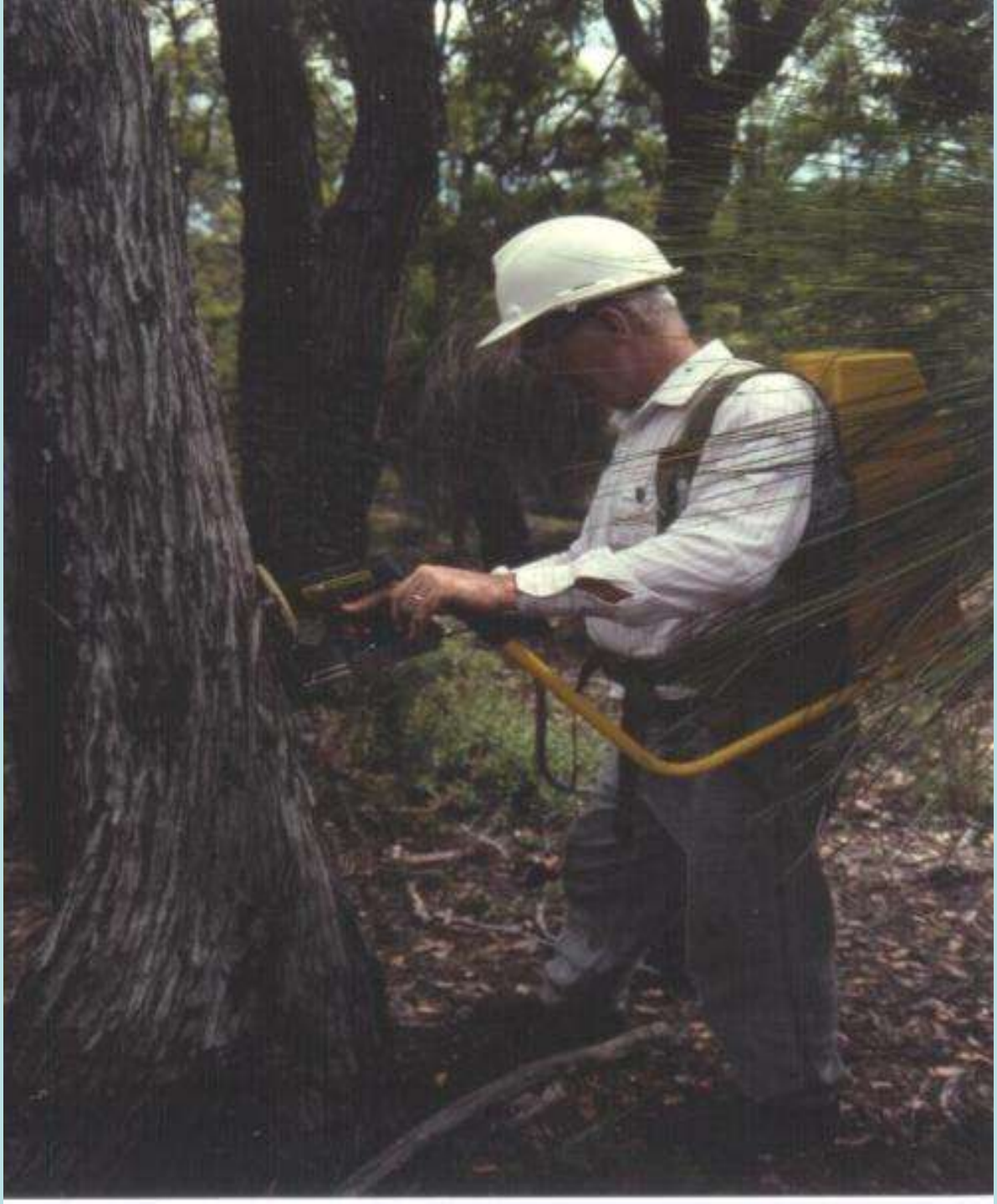
- What is disease-free material?
- False negatives using baiting and plating onto selective media
- Use of wetting and drying techniques can give recoveries in 2 of every 10 plants sampled

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

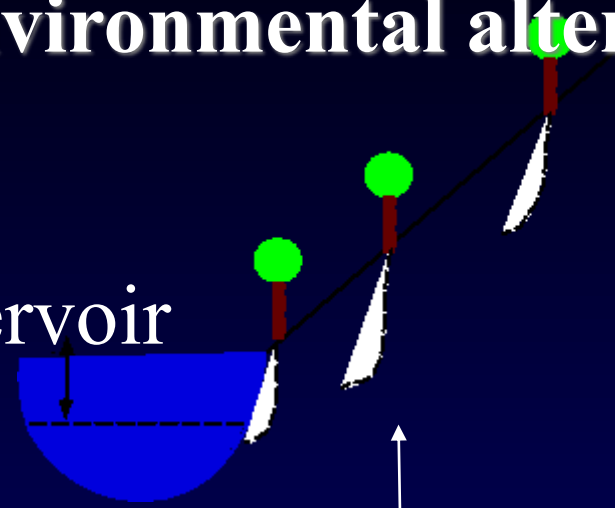
September Results:

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)

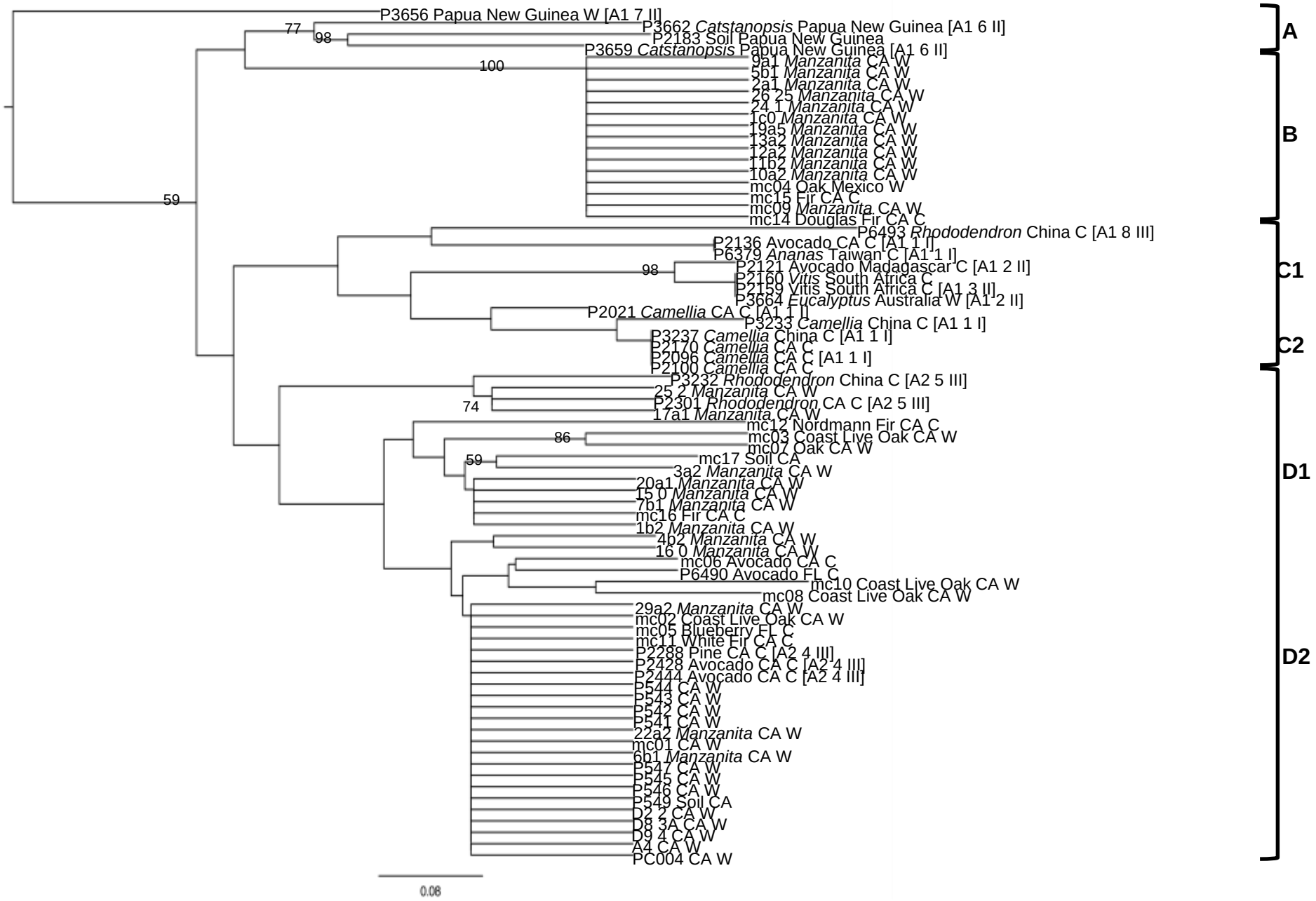
Ione manzanita is a rare endemic species of the Ione area, one that has well adapted to the local conditions, but it is currently in the list of US threatened species

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

How can we explain this diversity?

- At least four introductions of four 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


P3656 Papua New Guinea W [A1 7 II]
P3662 Catstanopsis Papua New Guinea [A1 6 II]
P2183 Soil Papua New Guinea
P3659 Catstanopsis Papua New Guinea [A1 6 II]
9a1 Manzanita CA W
5b1 Manzanita CA W
2a1 Manzanita CA W
26 25 Manzanita CA W
24 1 Manzanita CA W
1c0 Manzanita CA W
19a5 Manzanita CA W
13a2 Manzanita CA W
12a2 Manzanita CA W
11b2 Manzanita CA W
10a2 Manzanita CA W
mc04 Oak Mexico W
mc15 Fir CA C
mc09 Manzanita CA W
mc14 Douglas Fir CA C
P6493 Rhododendron China C [A1 8 III]
P2136 Avocado CA C [A1 1 I]
P6379 Ananas Taiwan C [A1 1 I]
P2121 Avocado Madagascar C [A1 2 II]
P2160 Vitis South Africa C
P2159 Vitis South Africa C [A1 3 II]
P3664 Eucalyptus Australia W [A1 2 II]
P2021 Camellia CA C [A1 1 I]
P3233 Camellia China C [A1 1 I]
P3237 Camellia China C [A1 1 I]
P2170 Camellia CA C
P2096 Camellia CA C [A1 1 I]
P2100 Camellia CA C
P3232 Rhododendron China C [A2 5 III]
25 2 Manzanita CA W
P2301 Rhododendron CA C [A2 5 III]
17a1 Manzanita CA W
mc12 Nordmann Fir CA C
mc03 Coast Live Oak CA W
mc07 Oak CA W
mc17 Soil CA
3a2 Manzanita CA W
20a1 Manzanita CA W
15 0 Manzanita CA W
7b1 Manzanita CA W
mc16 Fir CA C
1b2 Manzanita CA W
4b2 Manzanita CA W
16 0 Manzanita CA W
mc06 Avocado CA C
P6490 Avocado FL C
mc10 Coast Live Oak CA W
mc08 Coast Live Oak CA W
29a2 Manzanita CA W
mc02 Coast Live Oak CA W
mc05 Blueberry FL C
mc11 White Fir CA C
P2288 Pine CA C [A2 4 III]
P2428 Avocado CA C [A2 4 III]
P2444 Avocado CA C [A2 4 III]
P544 CA W
P543 CA W
P542 CA W
P541 CA W
22a2 Manzanita CA W
mc01 CA W
6b1 Manzanita CA W
P547 CA W
P545 CA W
P546 CA W
P549 Soil CA
D2 2 CA W
D8 3A CA W
D9 4 CA W
A4 CA W
PC004 CA W
77
98
100
59
98
74
86
59
0.08
A
B
C1
C2
D1
D2


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

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

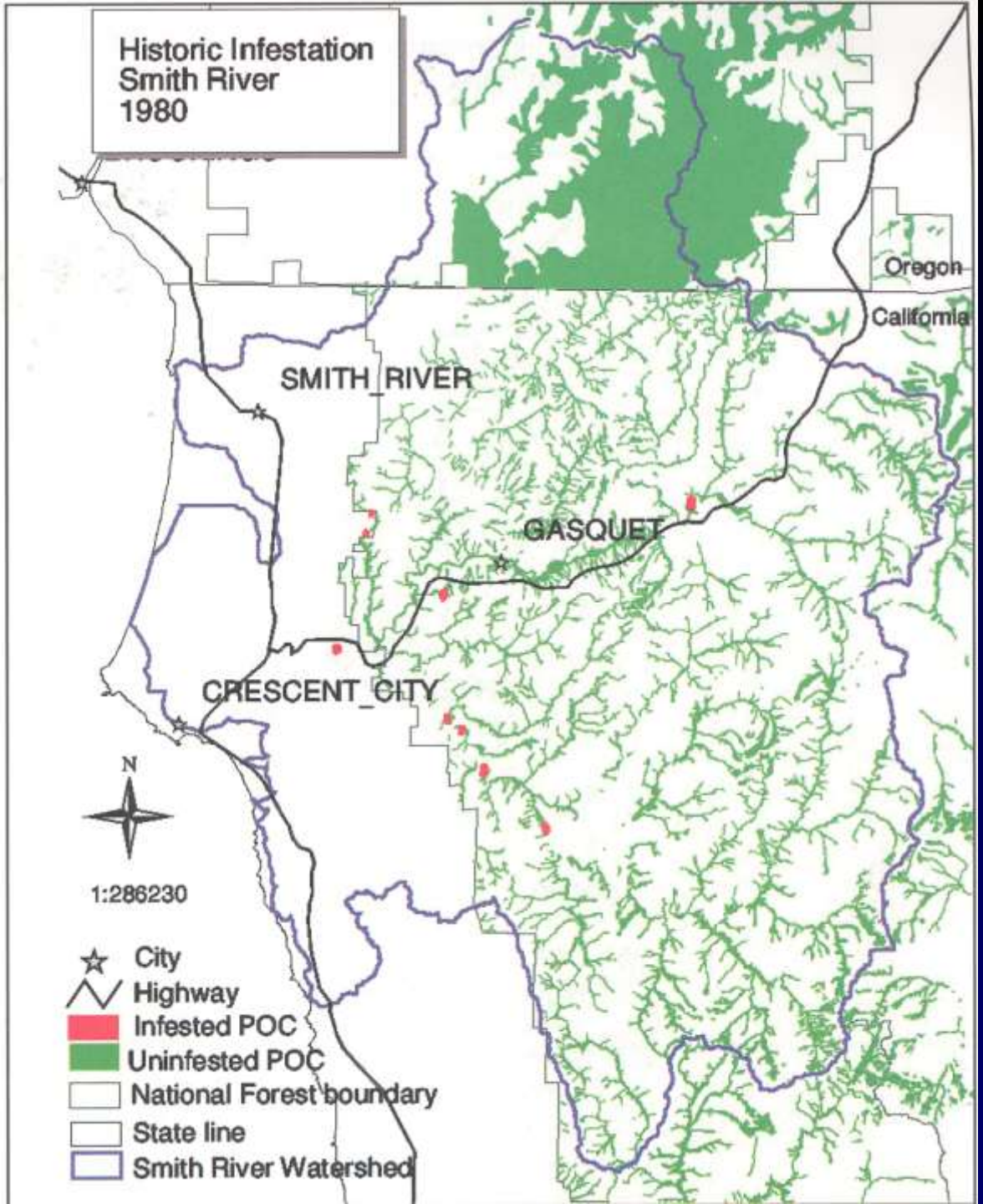

Photo: Paul Reeser

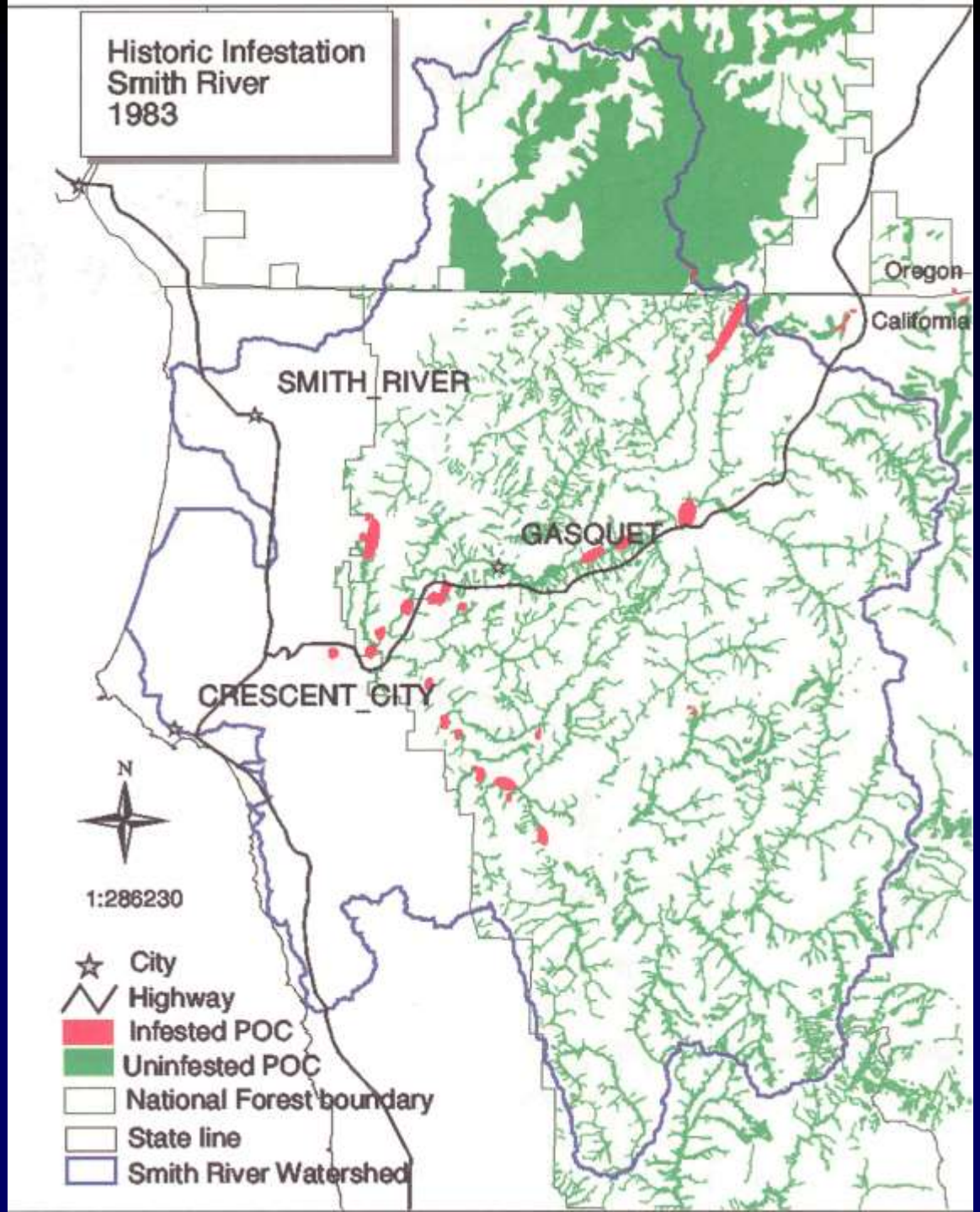
Port Orford cedar root disease

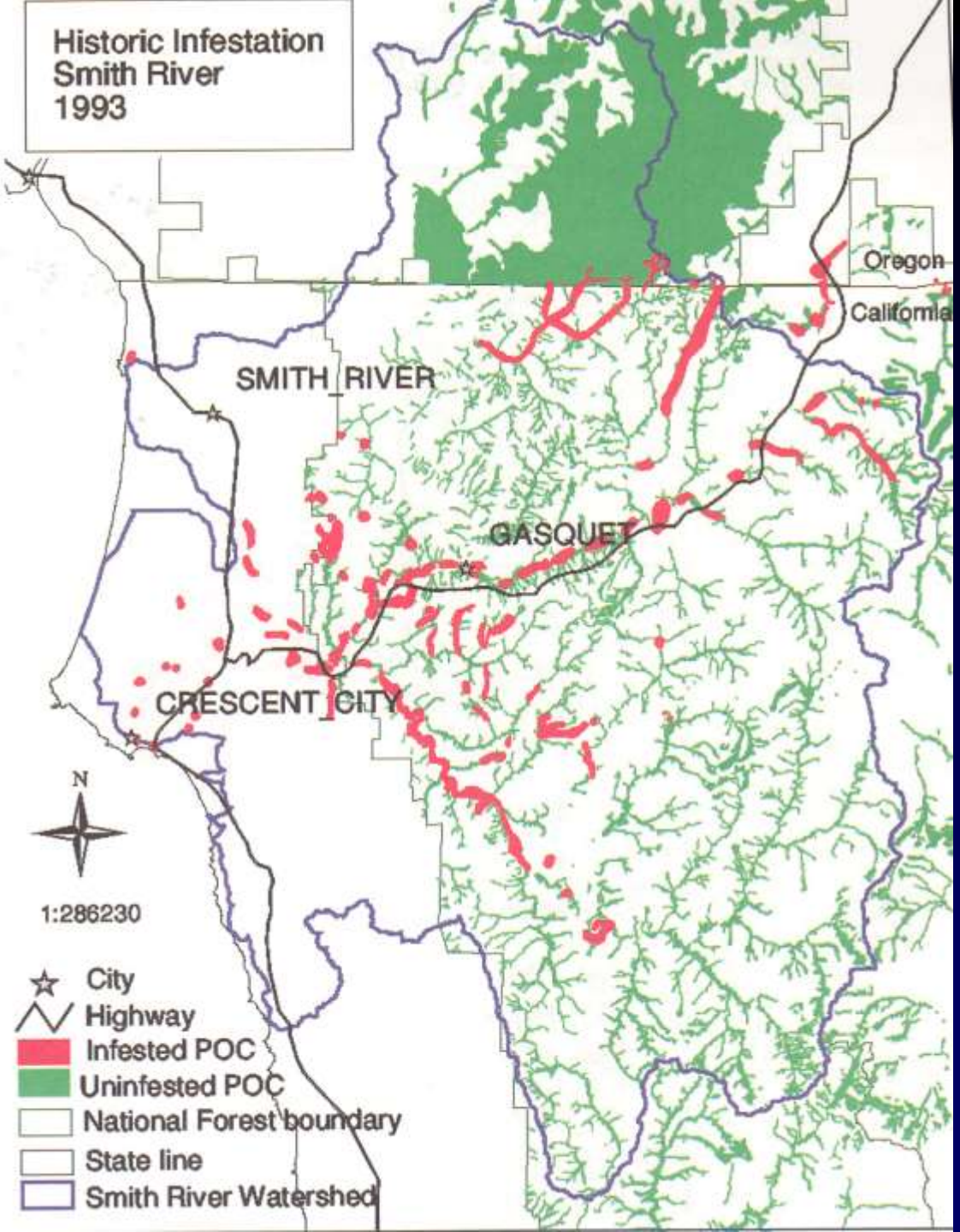
(*Phytophthora lateralis*)

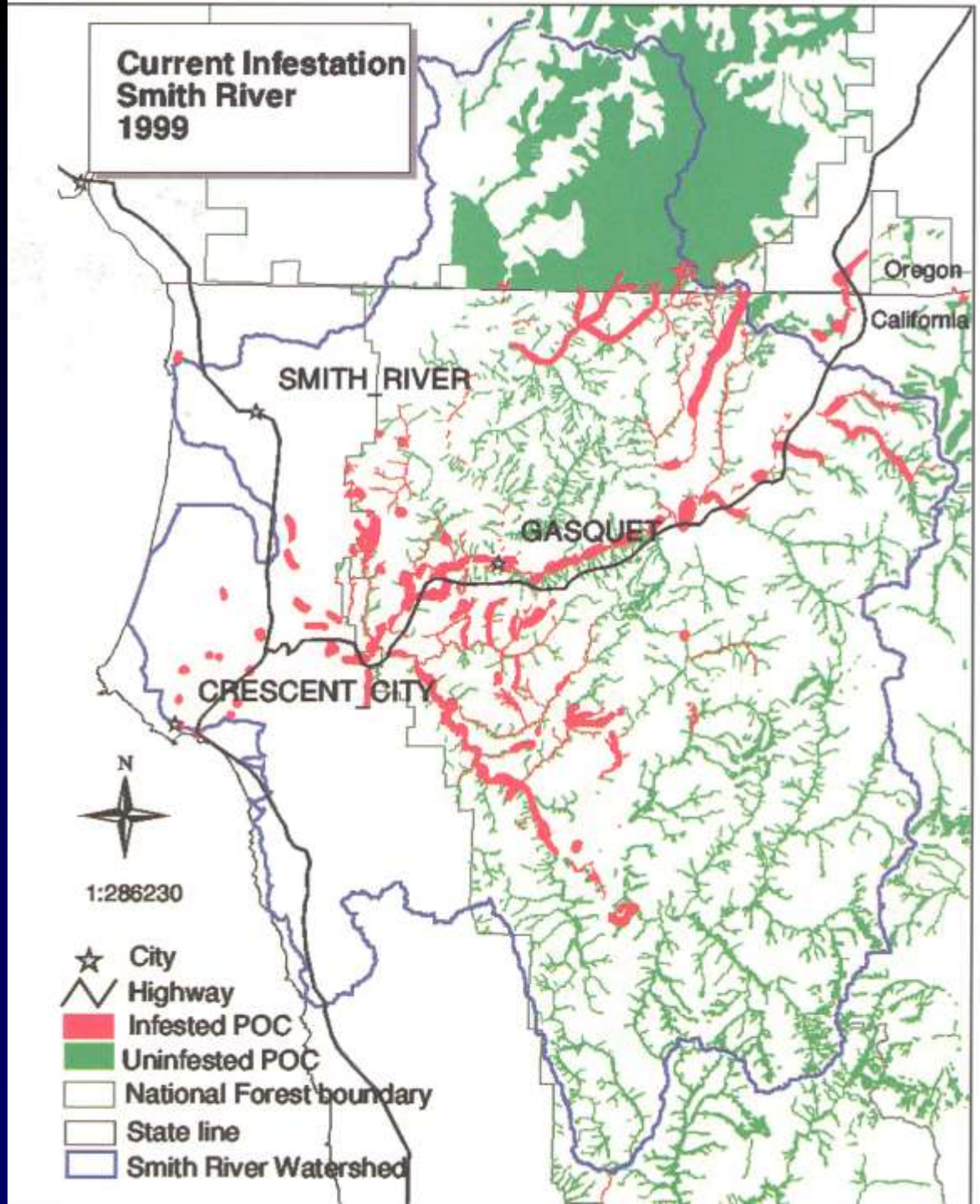
- Introduced in California from Oregon, to Oregon from Europe?
- Originally confined to plant nurseries
- Spread by soil and water movement
- Prevention by isolation possible
- Genetic resistance program

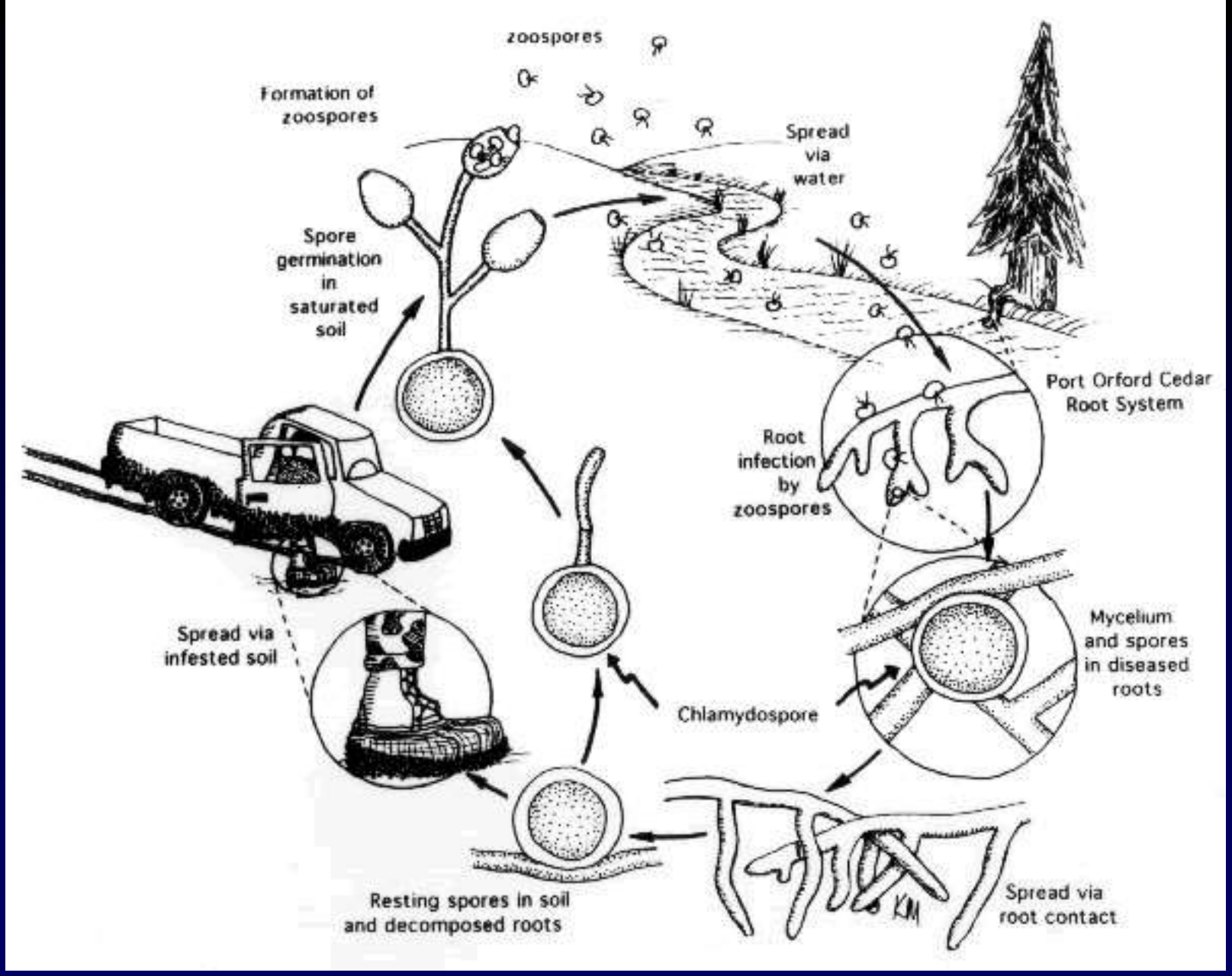
Association of mortality with roads and streams


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
Spread via infested soil
Chlamydospore
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

Where does it originate from?

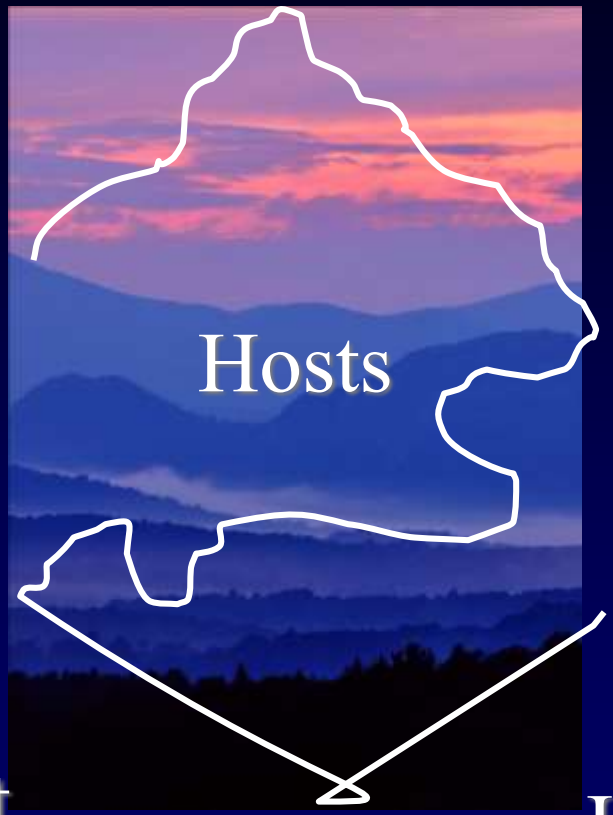
- Recently, *P. lateralis* isolated in Taiwan where local cedars display considerable resistance
- Although a clear adaptation of *P. lateralis* to be soil- and water-borne has been shown in the Pacific Northwest, there are recent reports of aerial infections of Port Orford Cedars from Europe, suggesting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

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

Sudden Oak Death: biology and management

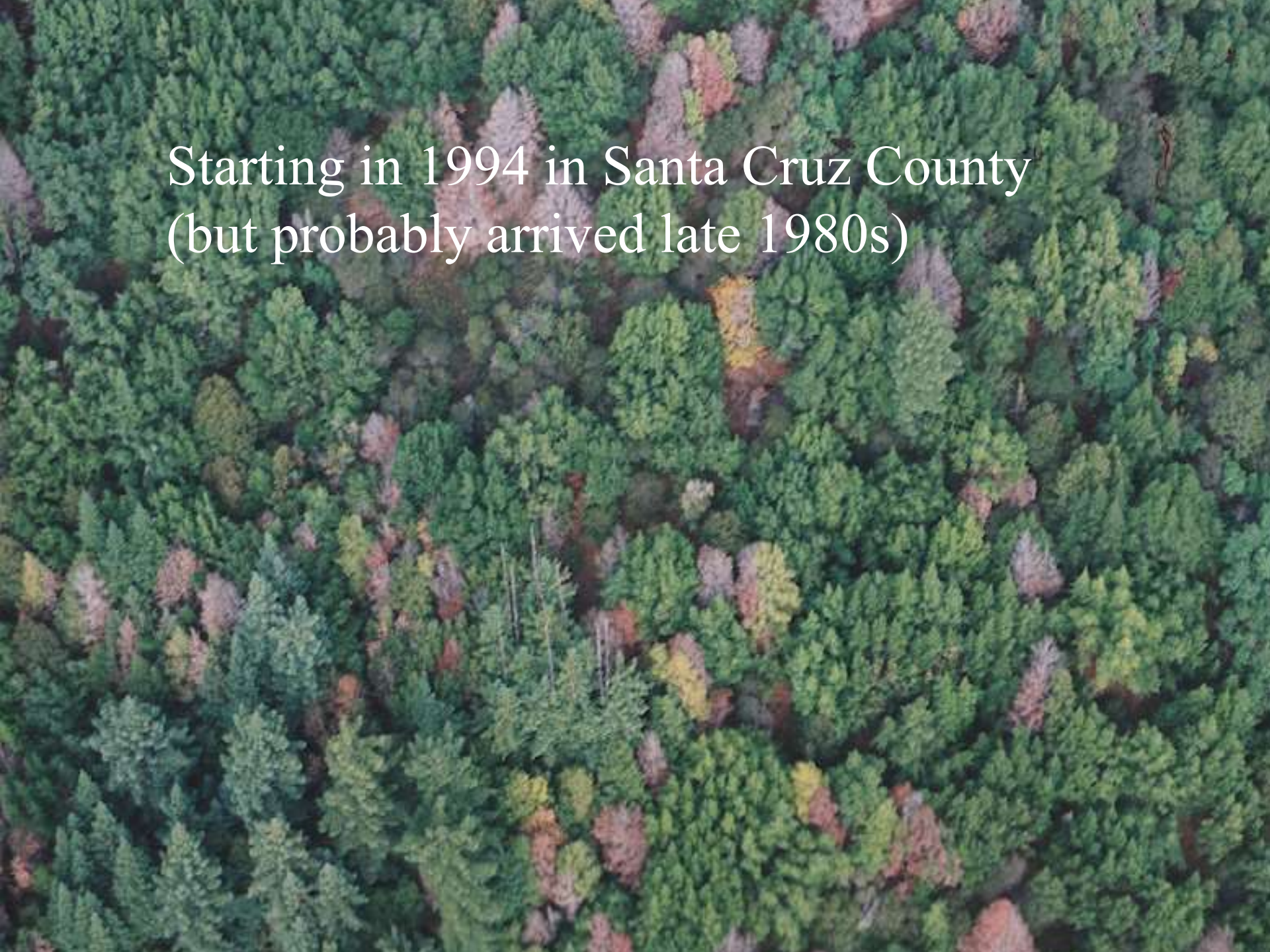
Pathogen

Man



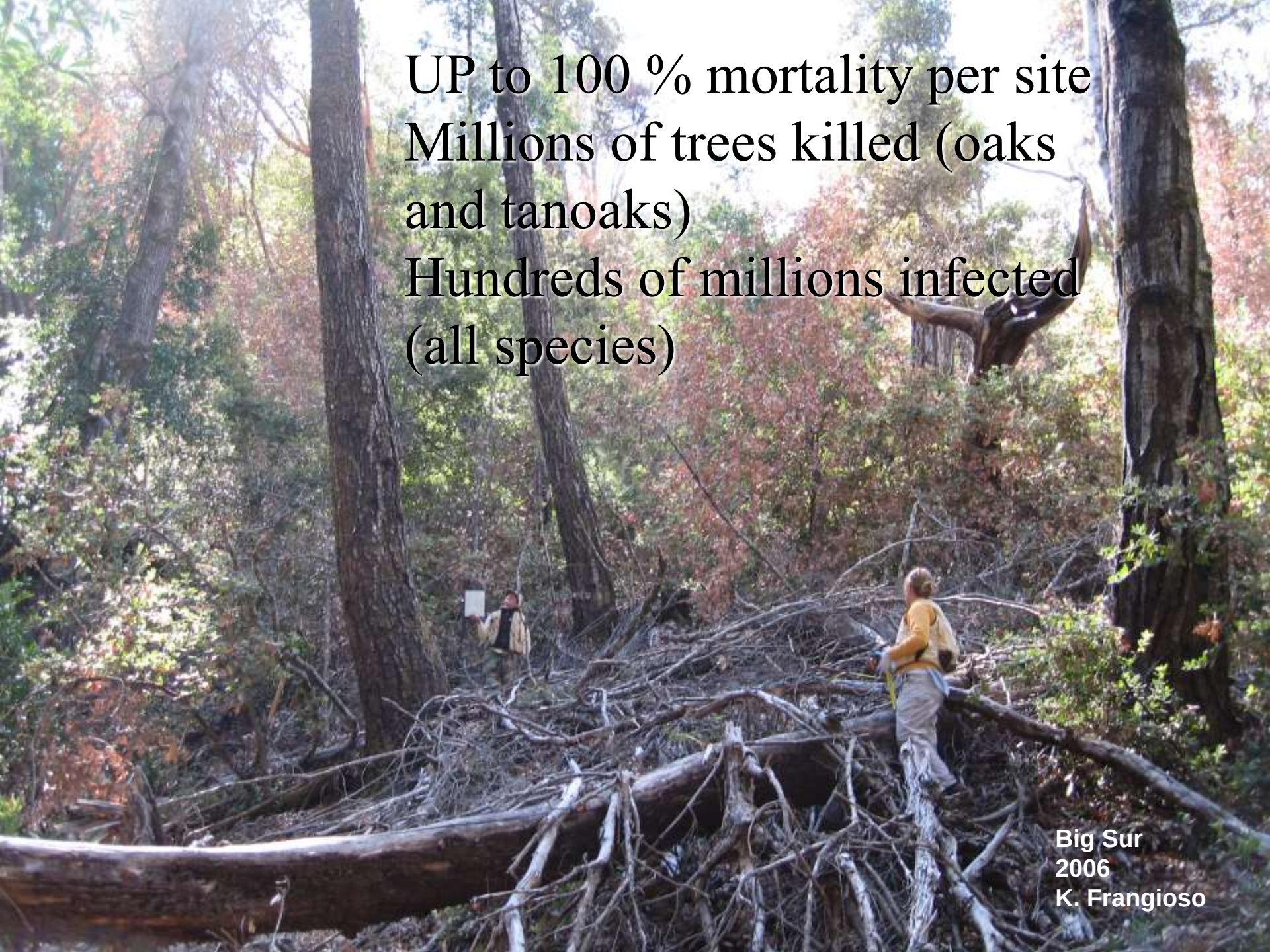
Environment

Interactions


Starting in 1994 in Santa Cruz County
(but probably arrived late 1980s)

2013 SOD damage, Broookings, OR

Girdling aerial ‘cankers’ removed from roots

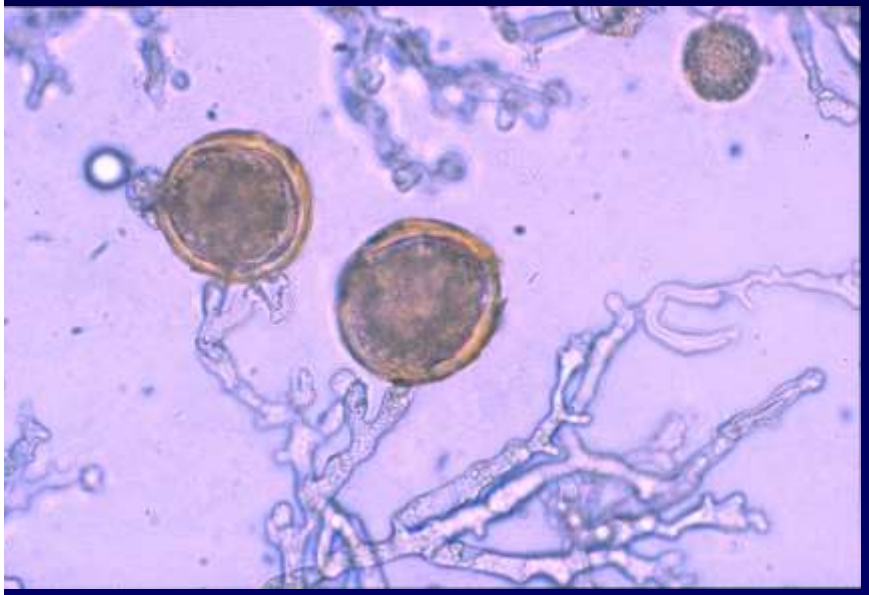

UP to 100 % mortality per site
Millions of trees killed (oaks and tanoaks)
Hundreds of millions infected (all species)
Big Sur
2006
K. Frangioso

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

Sporangia



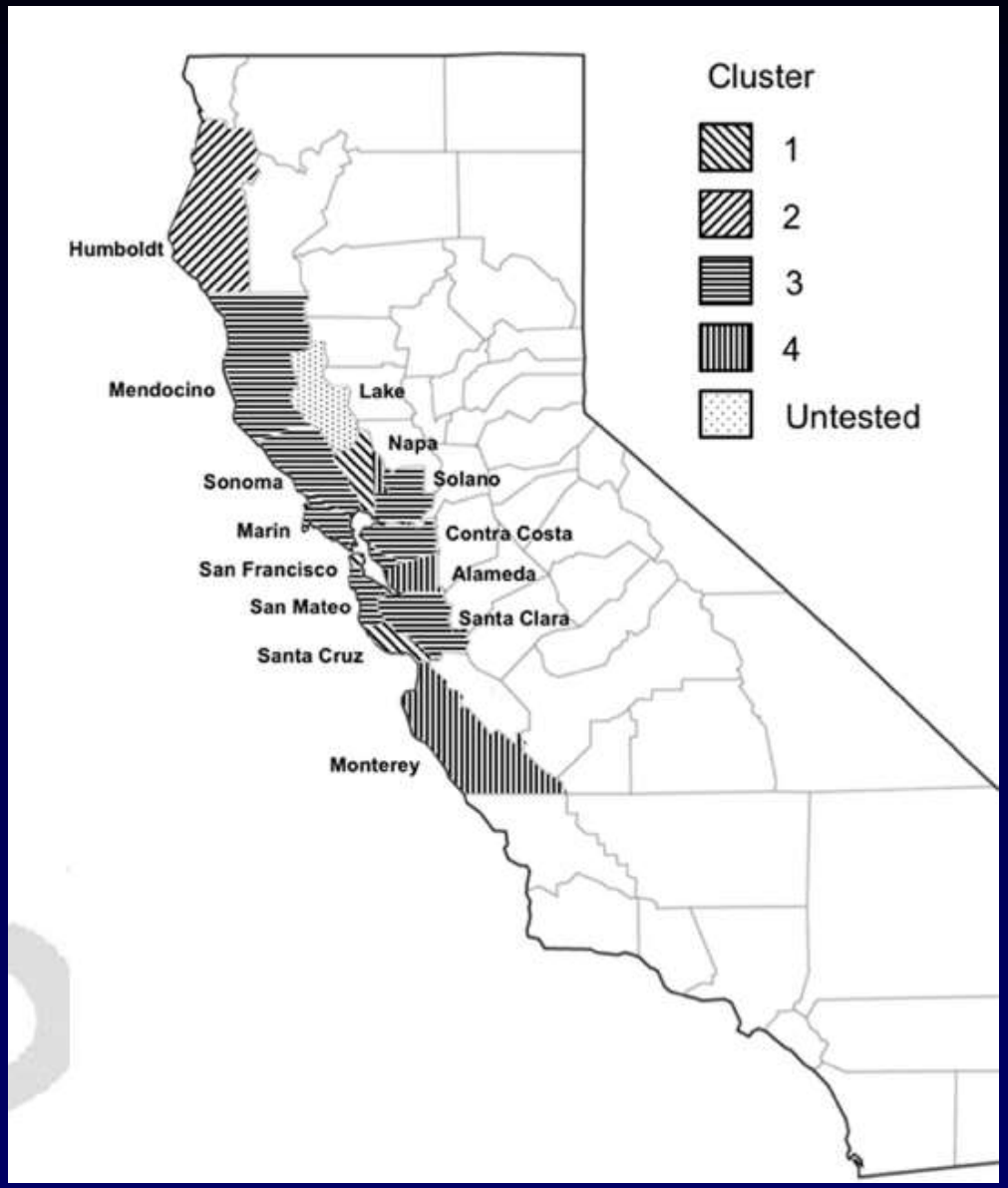
Chlamydospores

SOD pathogen called *Phytophthora ramorum* arrived to California in 80s on infected ornamental plants


Known distribution of
Phytophthora ramorum in
August 2008
SODMAP.ORG
HUMBOLDT
MENDOCINO
LAKE
SONOMA
NAPA
SOLANO
CONTRA COSTA
SAN FRANCISCO
ALAMEDA
SAN MATEO
SANTA CLARA
SANTA CRUZ
MONTEREY
Positive isolation
P. ramorum

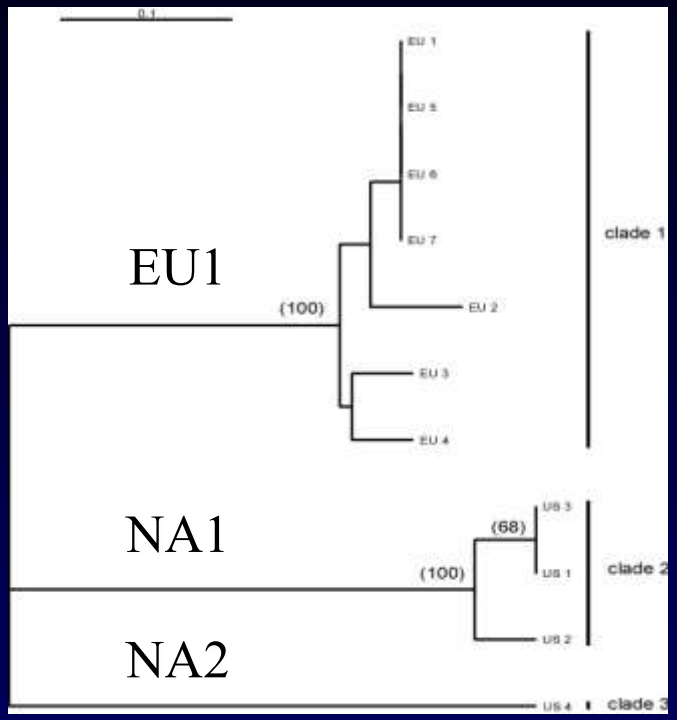
P. ramorum introduced at least 8 times in CA (Mascheretti et al. 2009). Multiple introductions and not ability to move far explain distribution of disease

Because pathogen is exotic, native flora has limited resistance to its attack and regular tree health maintenance simply will not suffice

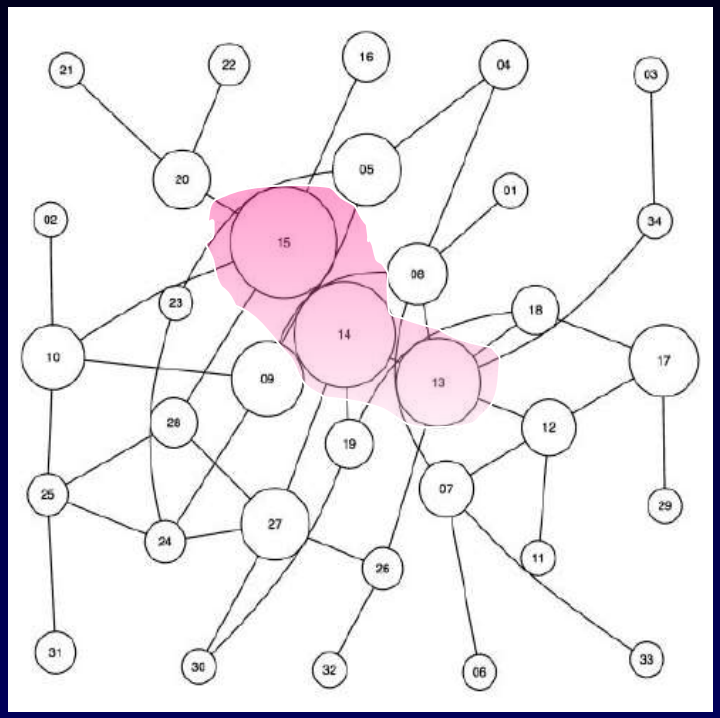


Cluster 1 of strains is the original introduced, but others are more widespread

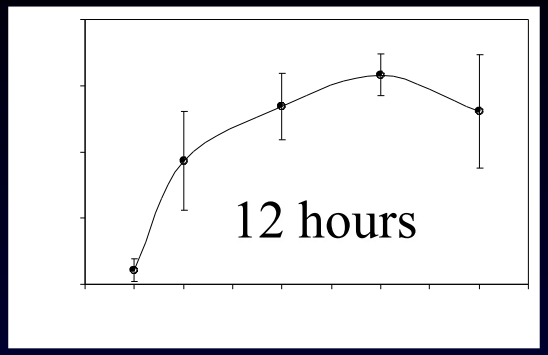
Pathogen

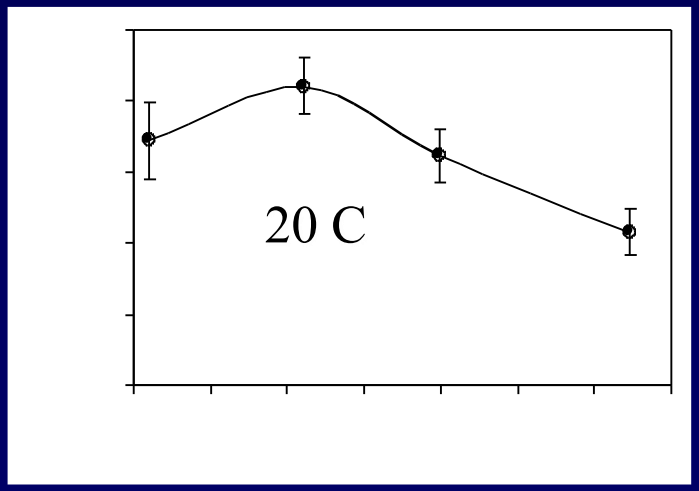


CA forests



Only one of three lineages introduced in CA forests (Ivors et al. 2007), but mutations in cyclically huge natural populations has increased the number of genotypes from three to over 100 , but all closely related (Mascheretti et al. 2008, 2009),. NA 2 genotypes distantly related





We tested the effect of:

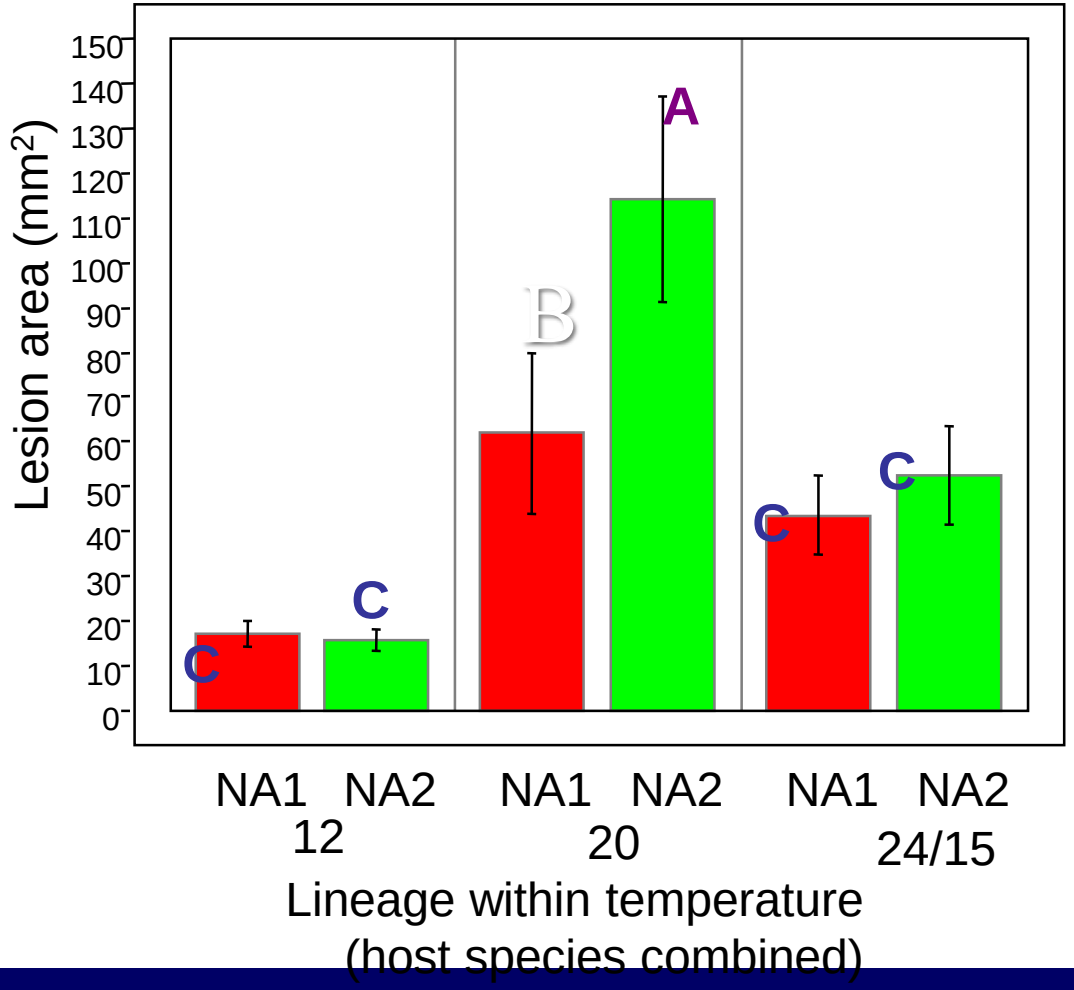
Lineage (NA1 vs NA2)

Temperature (12-20-24)

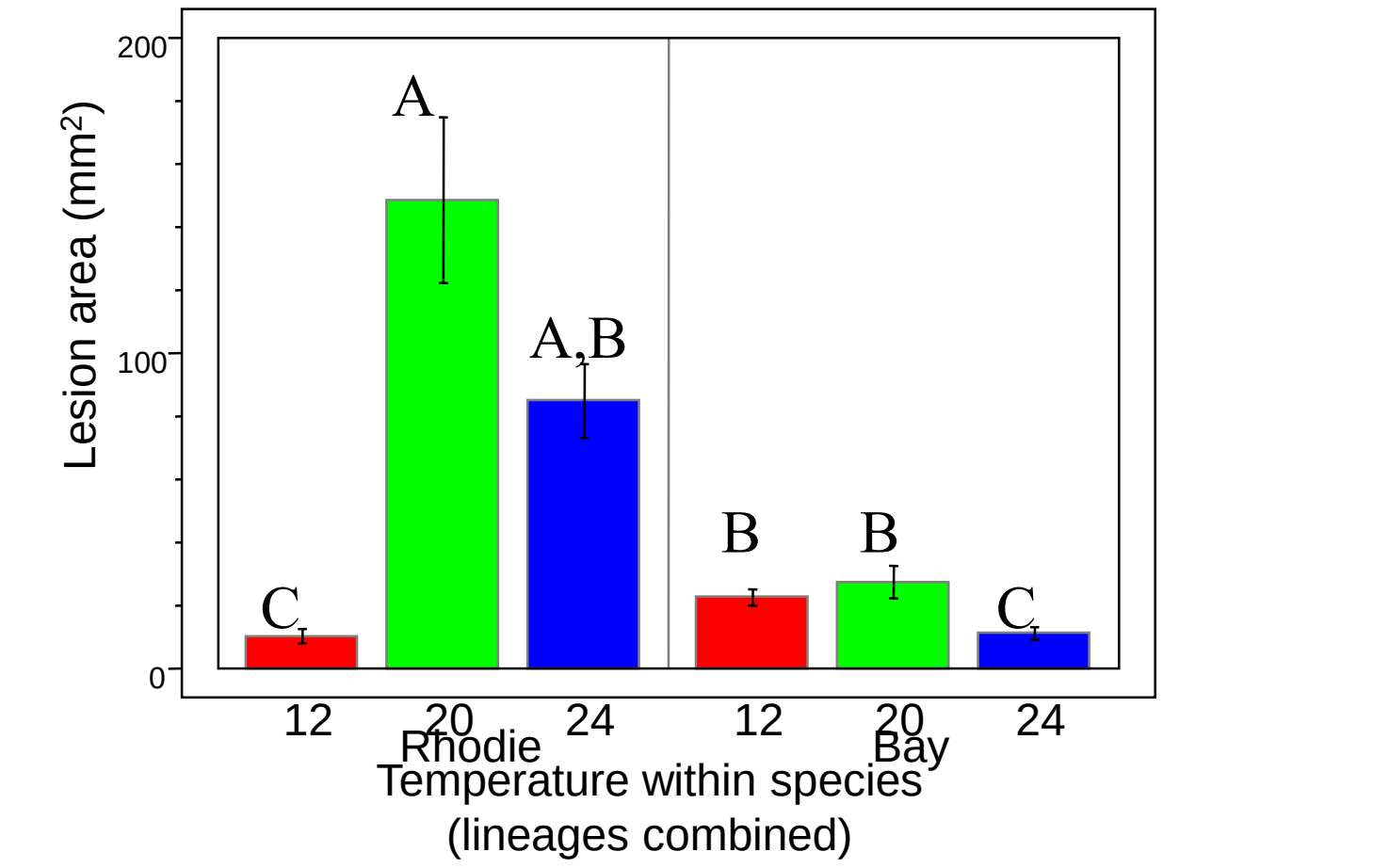
Host species (bay/ rhodie)

Method: detached leaves, zoospore inoculations, 5 genotypes per lineage but prescreened over 15 per lineage (Grunwald, Eyre, Kozanitas, Hayden)

NA2 performed better on each species at 20C; when we pool species, lesion size becomes significantly larger for NA2



In hot temperatures rhodies will develop large lesions, but bays will not, and vice versa. Bays would be good carriers where temperatures are colder than current ZOI, while rhodies would be good carriers where temperature are warmer than current ZOI



So who gave SOD to who?

- Genetic diversity much greater in nurseries with three evolutionary distinct lineages
- Only one lineage in forests, with little genetic diversity
- Individuals in nurseries and near nurseries are ancestral to all of forest population
- Nurseries are local source of pathogen, but escape of different lineages could be very serious (different mating type, and also different phenotype)

Distribution of SOD in California wildlands

- Distribution is result of discrete introductions followed by natural spread but only in favorable habitats (redwood-tanoak and mixed evergreen)
- As a result, distribution is extremely patchy in 14 contiguous coastal counties from Northern Humboldt to Southern Monterey
- Presence is extremely marginal in San Francisco, Solano, and Lake counties

Bay/Oak association

Bay

Yearly

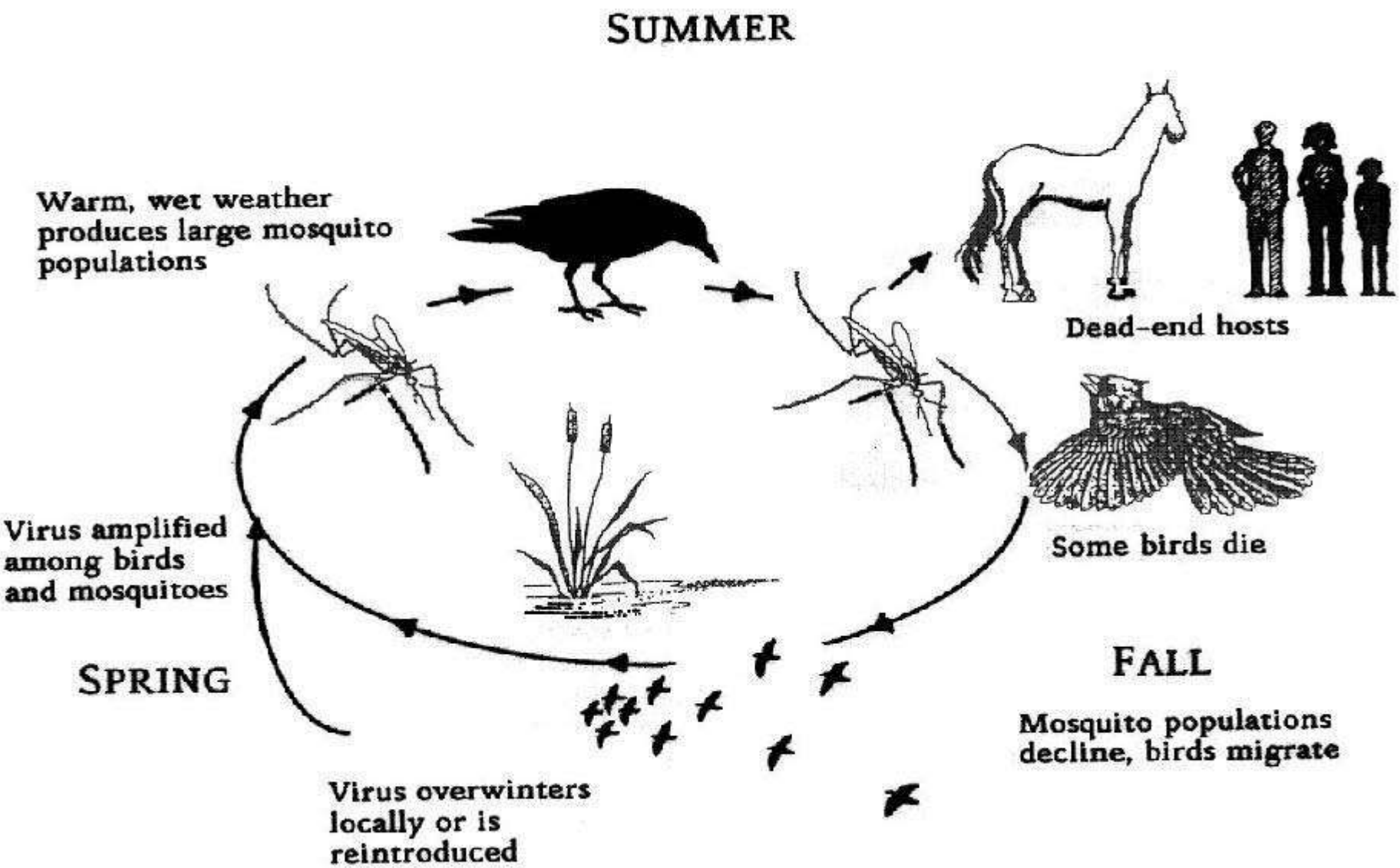
Coast Live Oak (no sporulation)

Sporangia

Wave years

Soil

Life Cycle of the West Nile Virus



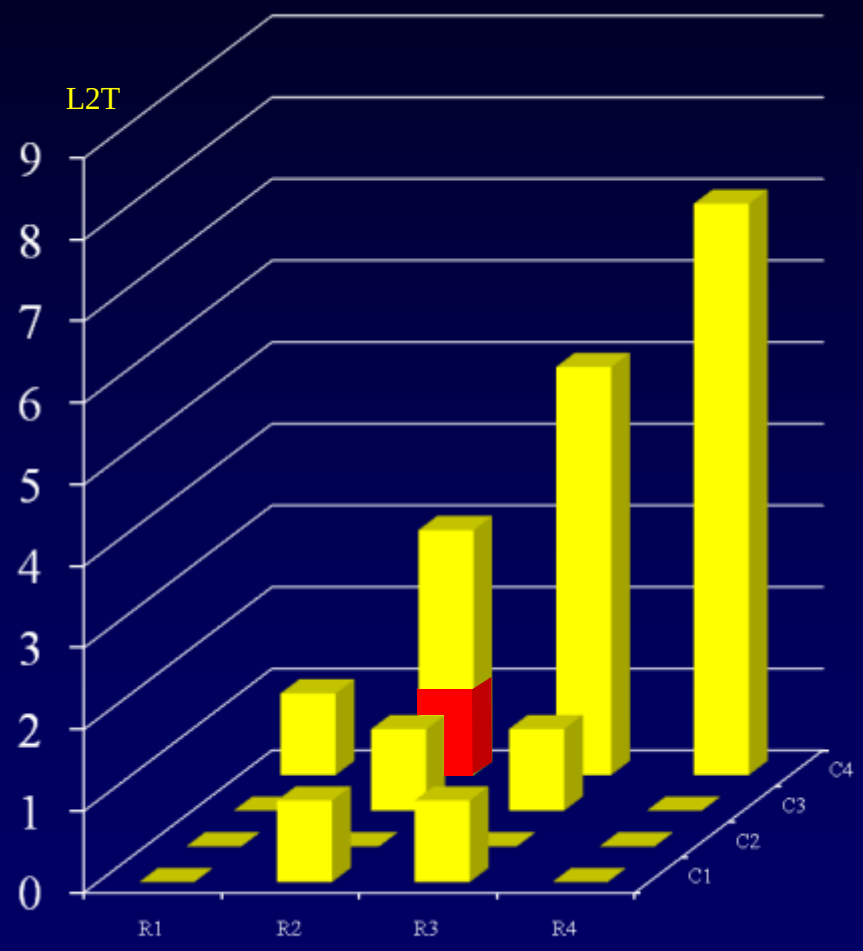
Landscape level factors

- Presence of sporulating hosts (bay laurels and tanoaks) positively correlated with mortality
- Madrone negatively correlated with mortality
- High diversity of hosts slowing down the disease
- Forest patches under 50 m not favorable
- Connectivity between stands (forest corridors) favors disease
- Because of recent arrival predictions are imperfect (lack of equilibrium)

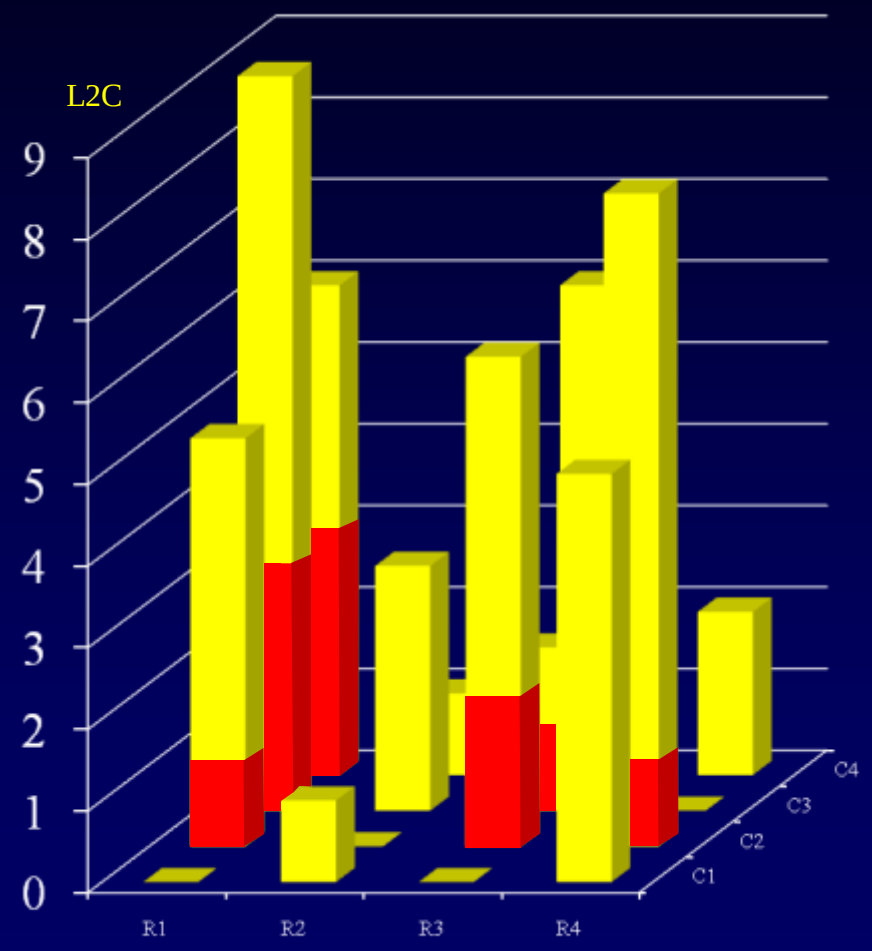
Bay Laurel Removal for SOD Control

Effect of Bay Removal on SOD Spore Counts

Combined *P. ramorum*
Spore Counts 2005& 2006



Bay Laurels Removed



Untreated Control

SOD Spore Monitoring at SDSF

8 Experimental Field Sites
16 Buckets / Site
5 Leaves / Bucket
= 640 leaves sampled every 3 weeks February through June for 6 years

Is reduction in spore loads caused by removal of bays in the 10-20 m range sufficient to prevent infection of oaks?

ONLY HIGH INOCULUM (RED) CAUSED OAK INFECTION

Conclusions:

1- Bay removal at moderate distance from oaks appears to sufficiently reduce inoculum level to prevent infection, even if it does not eliminate it Removal of bays 10 m around oaks recommended. For large specimens we recommend 20 m

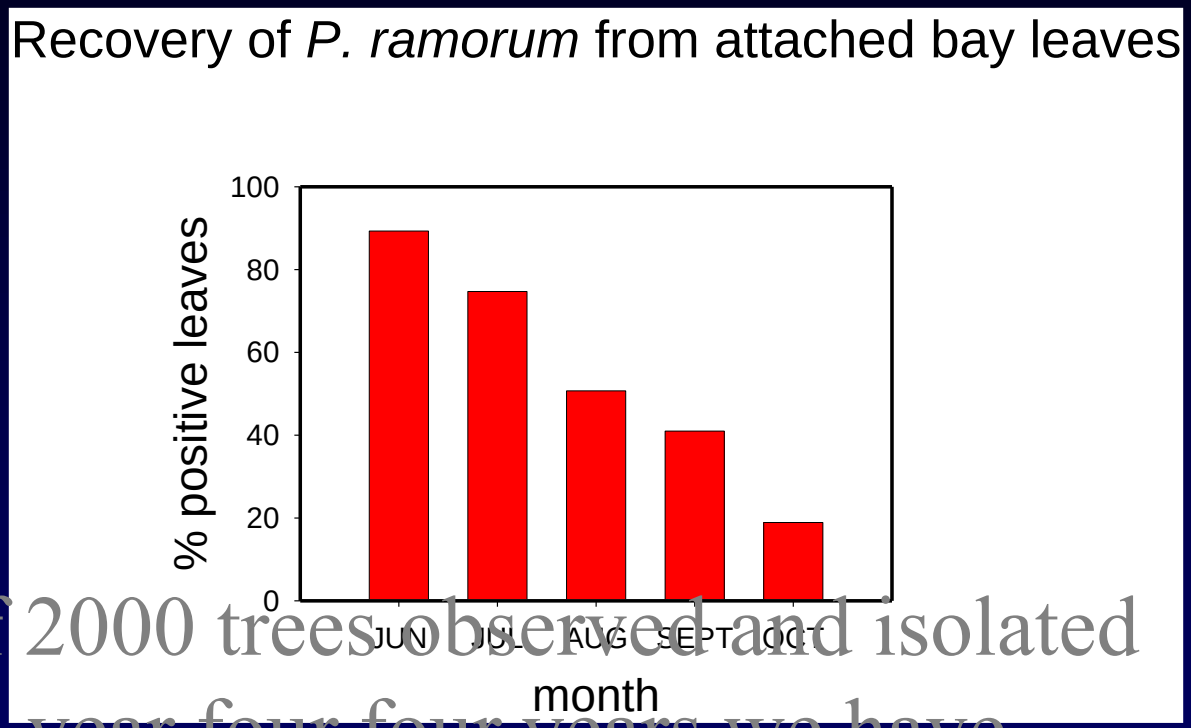
Oak-centric

2- Bay removal at the stand level will reduce inoculum. Floristically more diversified forests show lower disease incidence.

Stand-wide

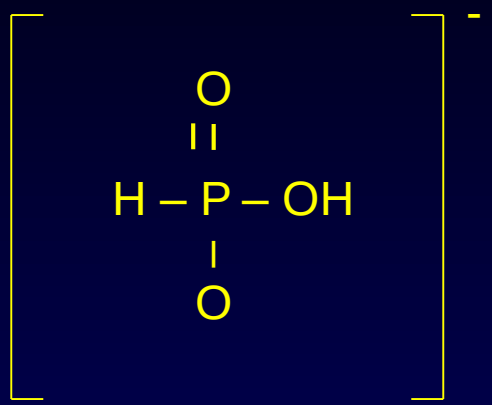
3- *P. ramorum* survives on bay leaves, but not everywhere. We have recently shown that only some sites allow survival during droughts. Elimination of bays in these sites will be very effective

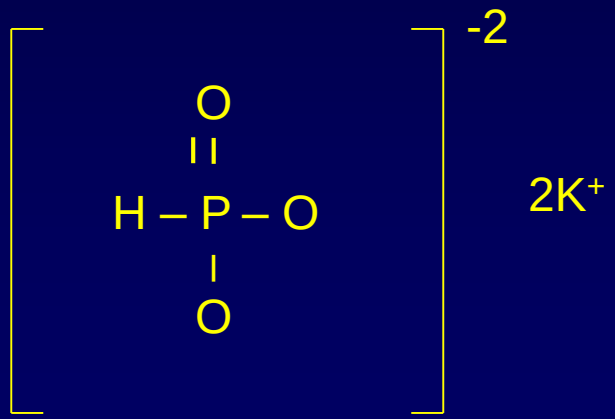
Ecology



In a survey of 2000 trees observed and isolated from 3 times a year four four years we have determined less than 5% of bay laurels carry over infection after dry season. We believe these 5% are key in epidemiology

Phosphonate (aka Phosphite) Chemical Treatments





- Water soluble. Systemically absorbed and translocated by the xylem and phloem
- Inhibits fungal growth and activates the plant's own defensive response
- Preventative treatments are more effective than curative


AGRI-FOS®
SYSTEMIC FUNGICIDE


AGRI-FOS


Injection Treatment

Plant Pathology (2008) Doi: 10.1111/j.1365-3059.2008.01894.x

Efficacy of phosphonic acid, metalaxyl-M and copper hydroxide against *Phytophthora ramorum in vitro* and *in planta*

M. Garbelotto*, T.Y.
Department of Environmental S

Arboriculture & Urban Forestry 33(5): September 2007 309
ISA
Arboriculture & Urban Forestry 2007. 33(5):309–317.
ARBORICULTURE & URBAN FORESTRY

Phosphite Injections and Bark Application of Phosphite + Pentrabark™ Control Sudden Oak Death in Coast Live Oak

M. Garbelotto, D.J. Schmidt, and T.Y. Hamik

Preventive treatment that strengthens response of oaks: we developed an alternative to injection

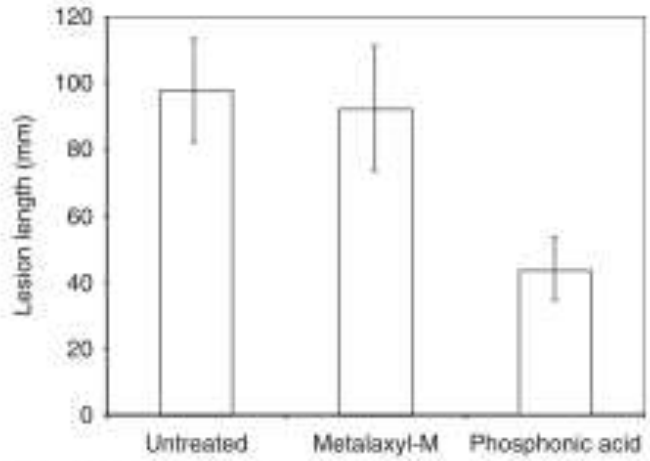


Figure 2 Lesion length (with bars showing standard deviation) caused by three *Phytophthora ramorum* isolates inoculated underbark in the phloem of potted coast live oak saplings, either untreated, treated with metalaxyl-M drench, or by phosphonic acid injection. Each treatment was performed on 15 saplings one week before inoculation; the experiment was terminated 6 weeks after inoculation.

Topical Treatment

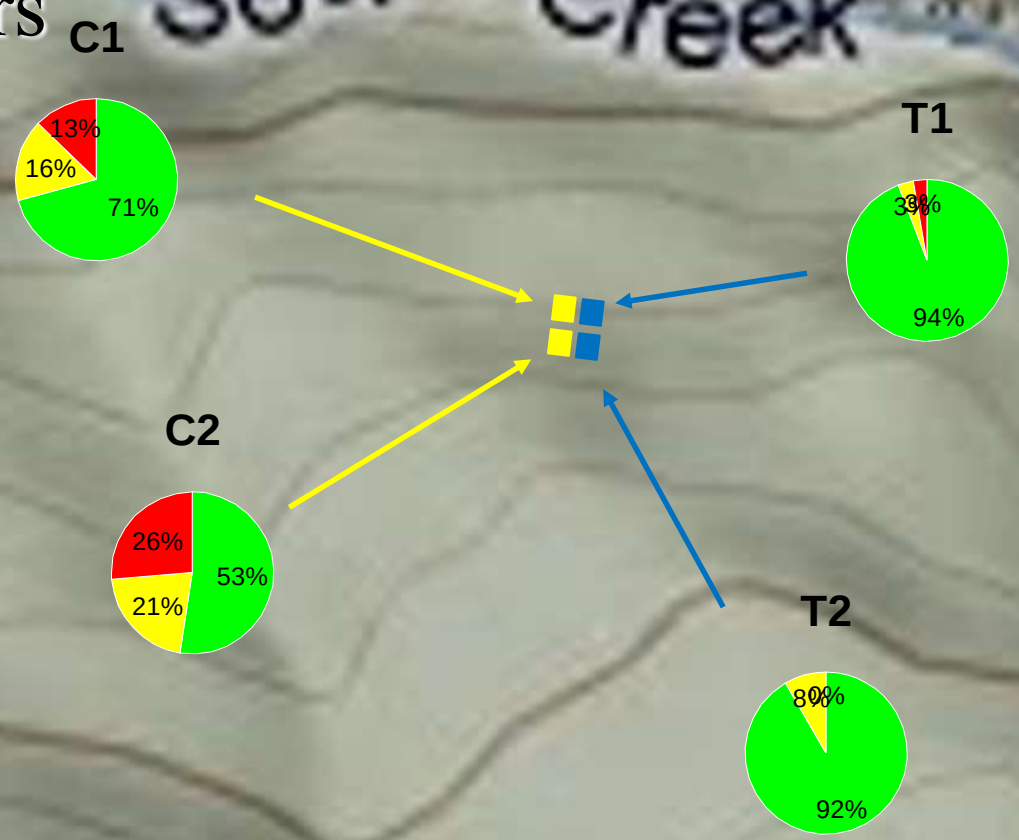
Long Term Treatment of Tanoaks SOD Spore Survey 2009

- 32 Field Plots
- 6 Sites in 3 Counties
- 672 Tanoaks > 8cm DBH

Long Term AF Treatment of Tanoaks

Treated 75 % healthy
Controls 56% healthy
32 plots in 16 pairs
P=0.0002

Year 3



Soquel Site A

Soquel Demonstration State Forest

11/2009

Bicycles for Cargo Transport

Conclusions on treatments

- Treat with phosphites before infection occurs (infected bays but oaks healthy/ entire tanoak cluster healthy)
- Treat once a year but in Fall to give time for plant to respond. If first treatment in Spring, repeat in Fall the first year. Do not treat in summer or December-January as trees do not respond well
- Injection holes will seal in three years, do not inject in spring as drill holes could facilitate infection

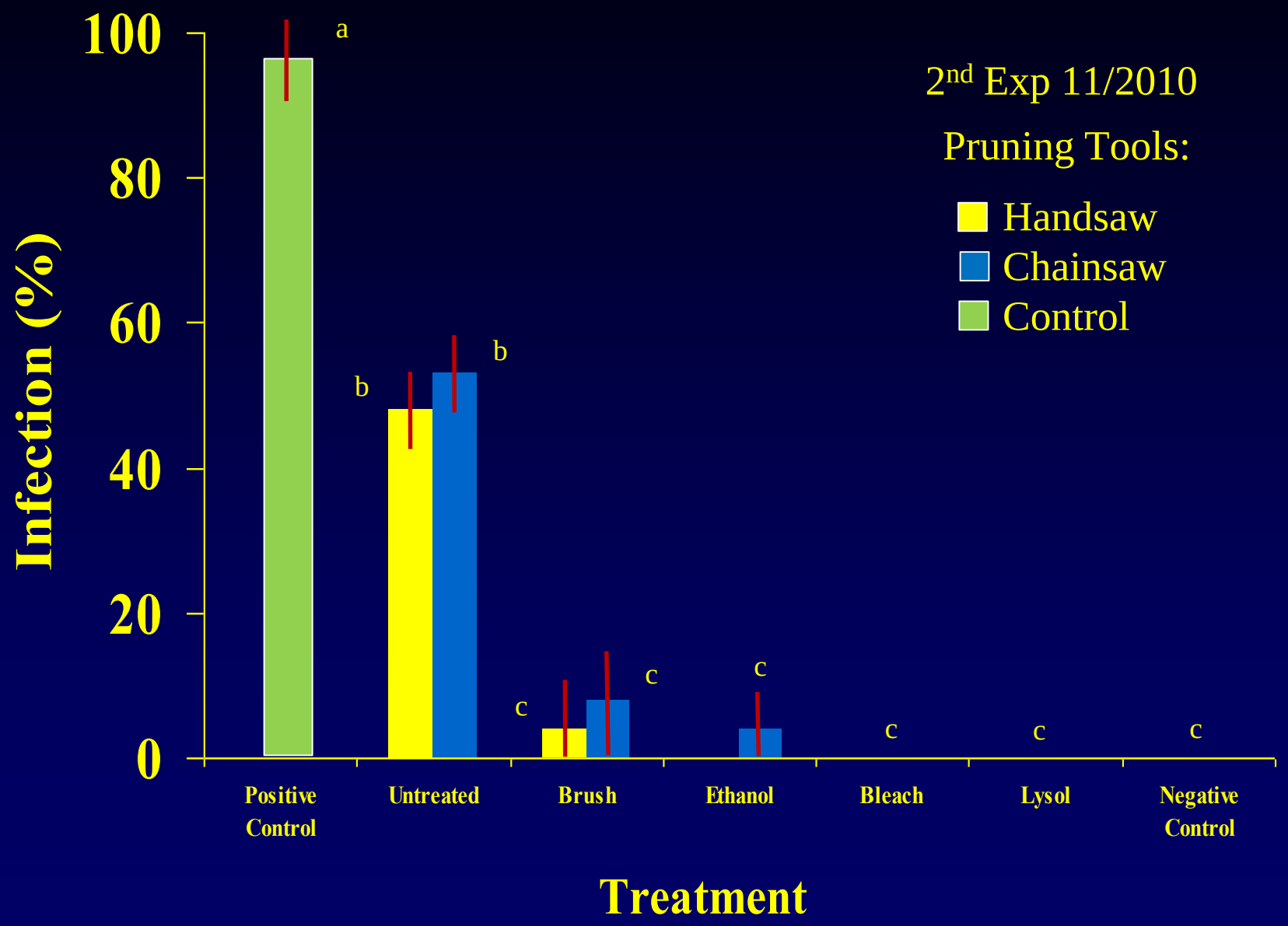
Four Treatments:

- Wire Brush
- 70% Ethanol + Brush
- 5% Bleach (Na Hypochlorite) + Brush
- 6.25% Lysol (ADBAC) + Brush

Cleaning Tools

Infected Tool Surface

Transmission of SOD Through Pruning Tools



Sanitation

Green waste more infectious than wood and soil

Drying infected material is best strategy to sanitize: small chips best, thin layers best, exposure to sunlight best, dry on site before removing if possible

For sanitation of equipment, tools, and vehicles: if it looks clean it is not infectious

The search for the Holy Grail of resistance:

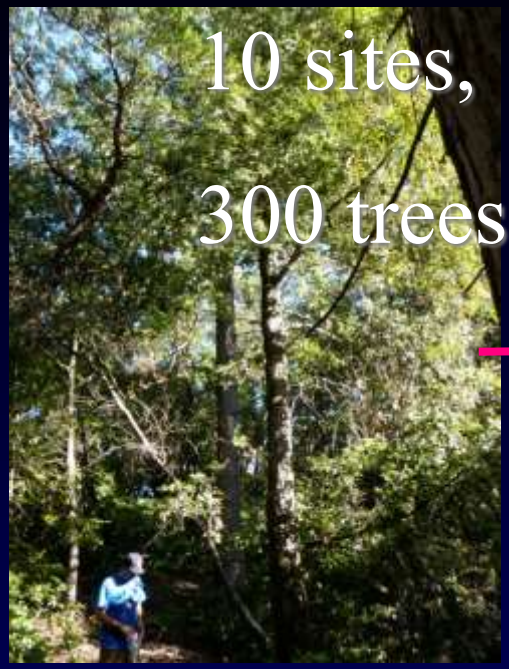
There are significant differences in susceptibility among individuals within all species tested

Constitutive chemistry and/or phenology invoked to explain differences that are both inheritable (i.e. genetic)and determined by the environment

Resistance proper not found yet, but decreased susceptibility and/or tolerance may be extremely useful and more durable

The search for the Holy Grail of resistance:

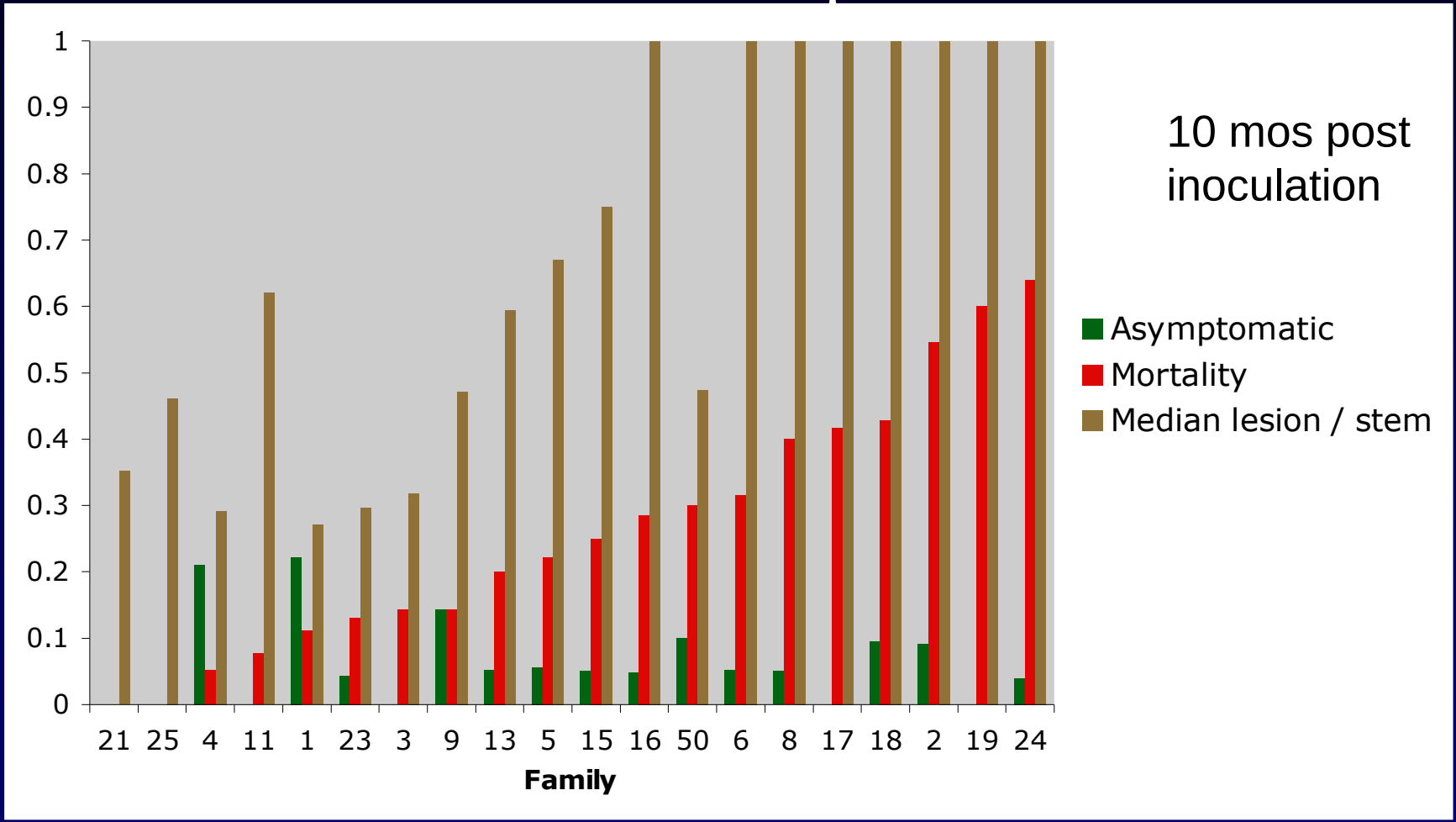
- Ongoing screening for resistance in tanoak includes common garden tests both in lab and nature. With phenotypic traits studied by family (half sibs) including lesion size, survival in absence and presence of SOD and morphology


10 sites,
300 trees

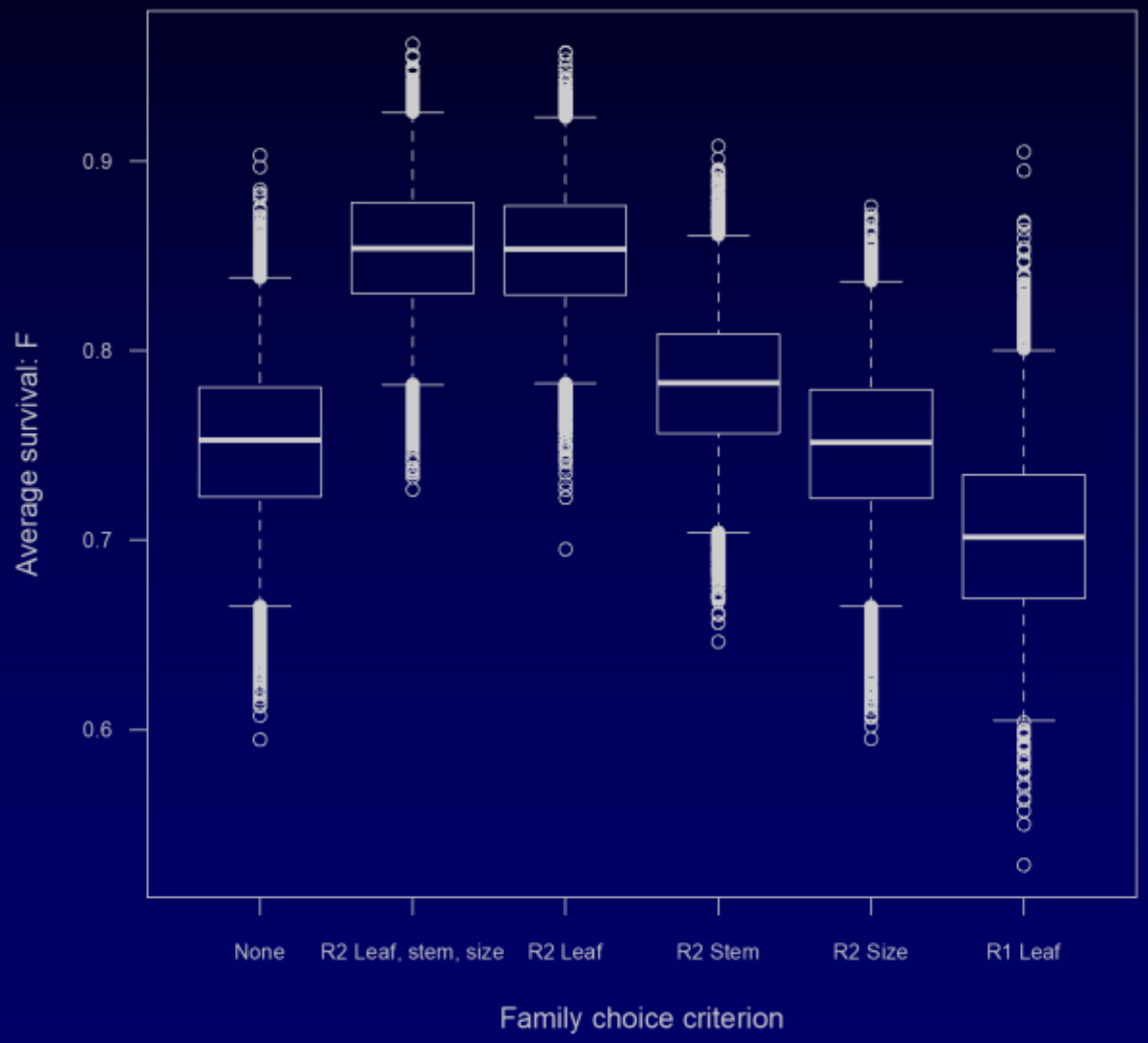

20,000


800

Common garden seedling tip assays of families indicates role of genetic variation within host species



Survival highest in families picked as more resistant based on lab assays



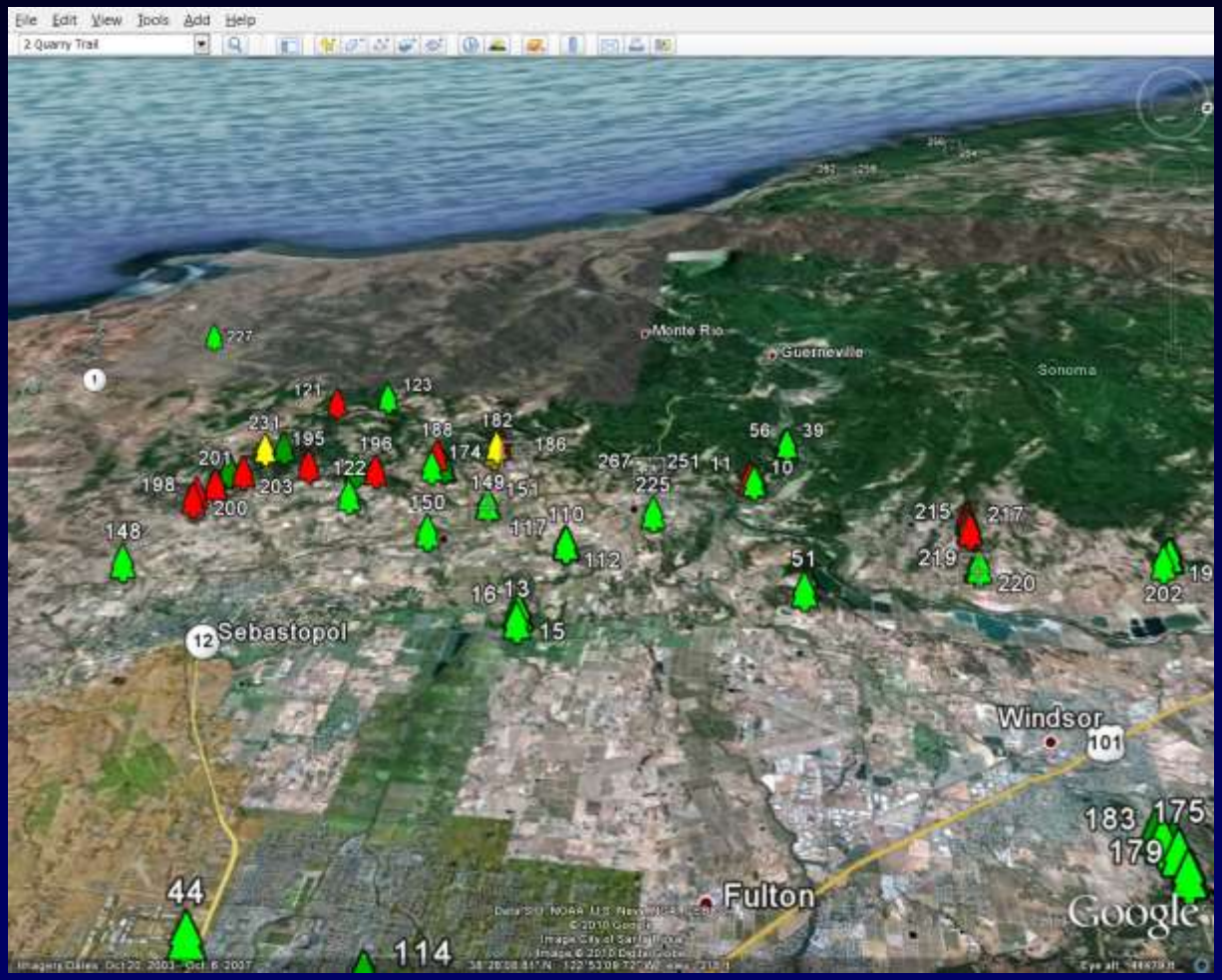
Why should we care about variation in susceptibility?

1- Less susceptible oaks/tanoaks in habitats less conducive to *P. ramorum*= survival

2- High susceptibility of bays can be used to predict sites with the highest risk of SOD outbreaks

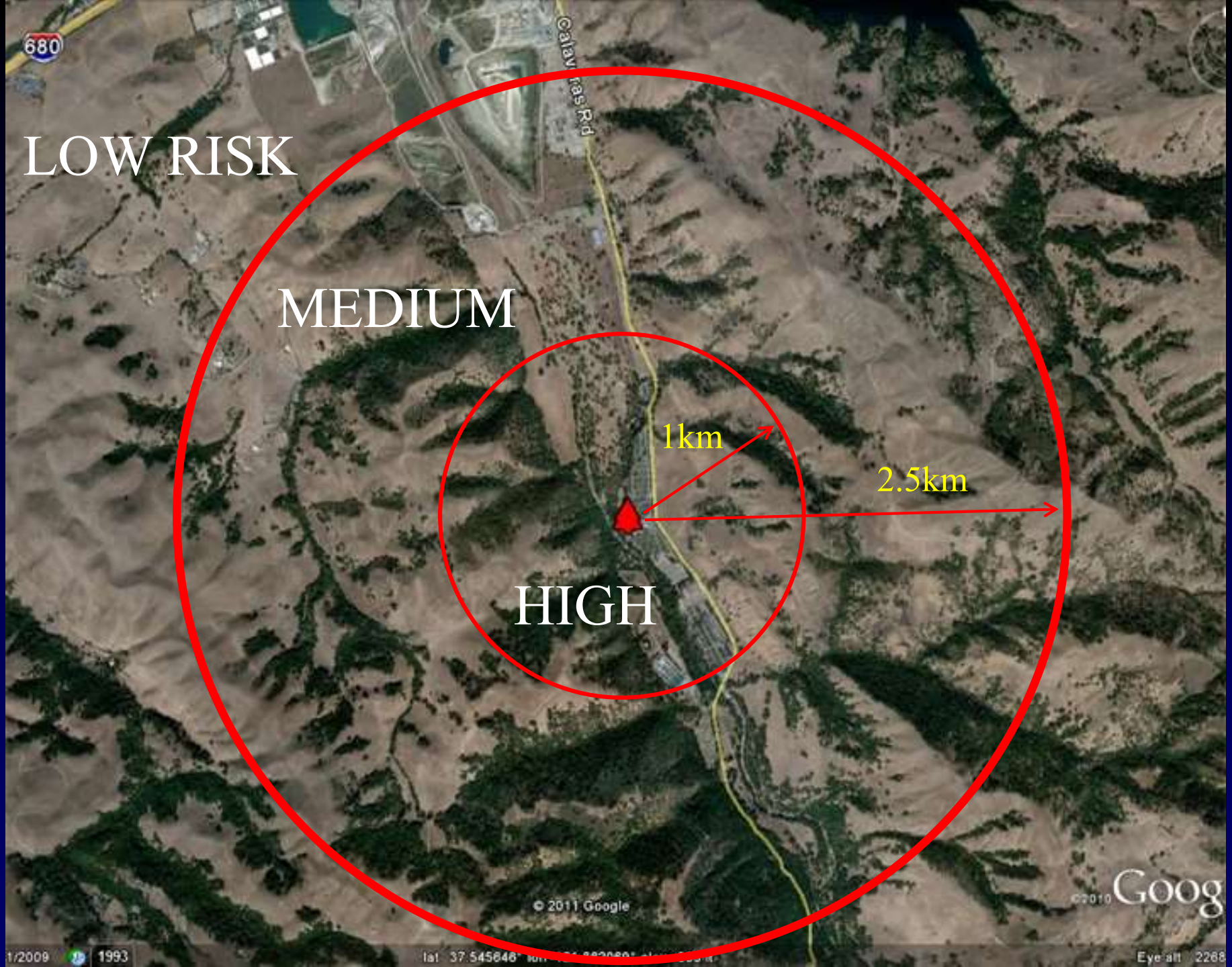
3- We have shown that reforestation efforts using families that show low susceptibility in the lab and good growth are going to be significantly more successful

SOD Blitzes and SODMAP (June 2012)
www.sodblitz.org



What have we learned from Blitzes

- Disease incidence triples during rainy year even in old infestations
- New infestations discovered
- In truly coastal areas, disease incidence remains high, while in more interior areas there are significant fluctuations (e.g. Western vs. Central Sonoma)
- Spread rate will change when disease changes climatic zone


LOW RISK
MEDIUM
HIGH
1km
2.5km
680
© 2011 Google

What is the

SODMAP Project

- The SODMAP Project is a partnership of scientists and citizens, working together to create the most complete distribution map of a forest disease ever produced in the world.

- SODMAP incorporates laboratory confirmed collections of plant and

Sodmap.org

home
research
publications
diagnosis
treatment
links
contact us
ucb courses

Featured
This Month

SODMAP Project

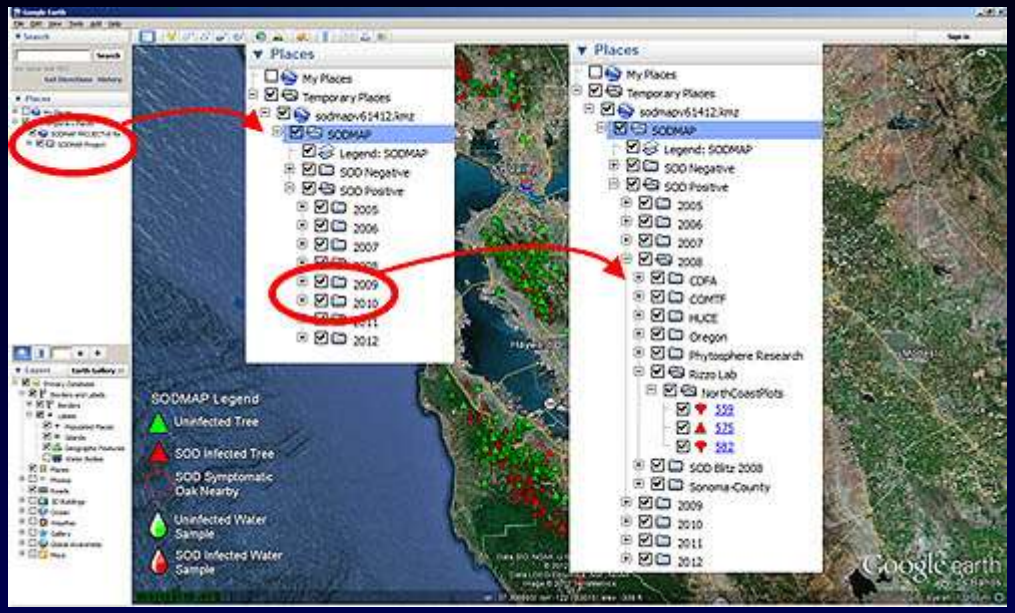
This project made possible thanks to funding from:
USDA Forest Service, State and Private Forestry
The Gordon and Betty Moore Foundation

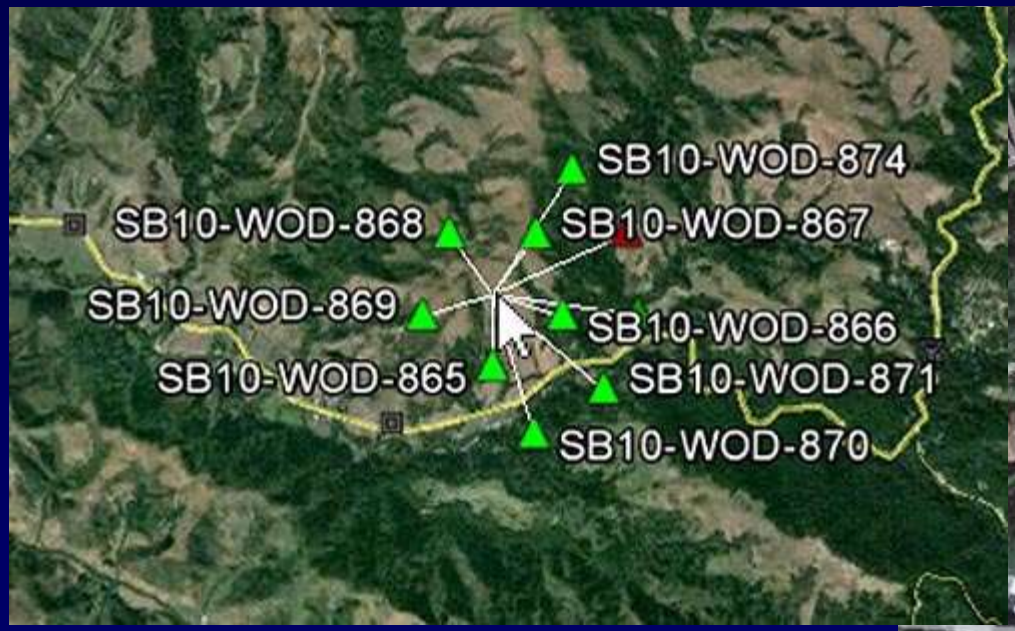
SODMAP Guide and Instructions

The SODMAP Project is a partnership of scientists and citizens, working together to create the most complete distribution map of a forest disease ever produced in North America. SODMAP incorporates laboratory confirmed collections of plant and water samples from 2005 to the present. It includes both SOD-positive, as well as, SOD-negative specimens to better illustrate the range and distribution of the disease.

SODMAP is the result of a collaboration between hundreds of citizen scientists participating each year in the SOD Blitzes organized by the U.C. Berkeley Forest Pathology and Mycology Laboratory, other research organizations, and government facilities. Contributors to the 2012 SODMAP include: M. Garbelotto, UC Berkeley; D.M. Rizzo, U.C. Davis; Ross Meetenmeyer; UNC Charlotte, Ted Swiecki, Phytosphere Research; Don Owen, Cal Fire; Jack Marshall, Cal Fire; Cheryl Blomquist, CDFA; Lisa Bell, UCCE; Yana Valachovic. UCCE.

Using SODMAP





Big Sur fires 2008

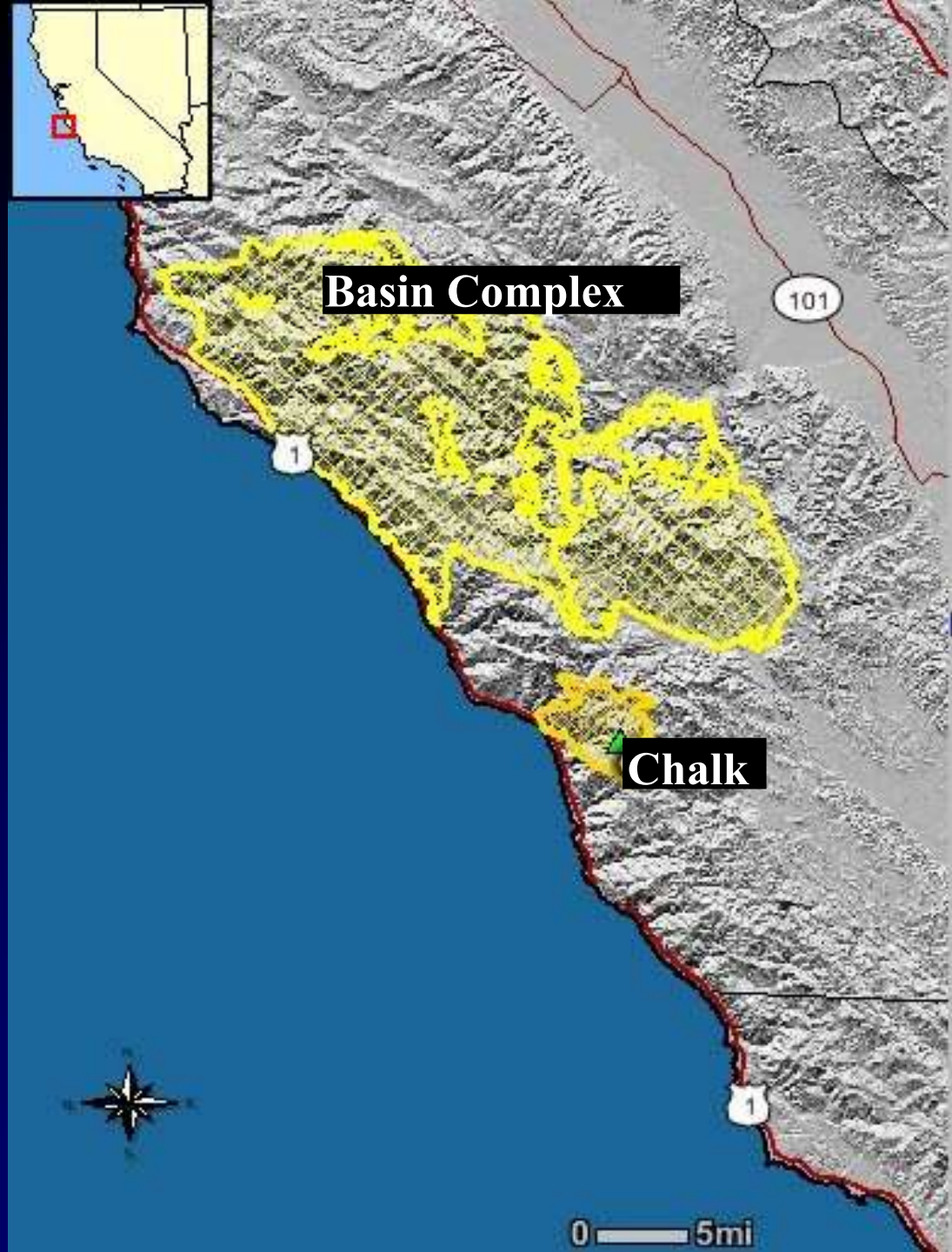


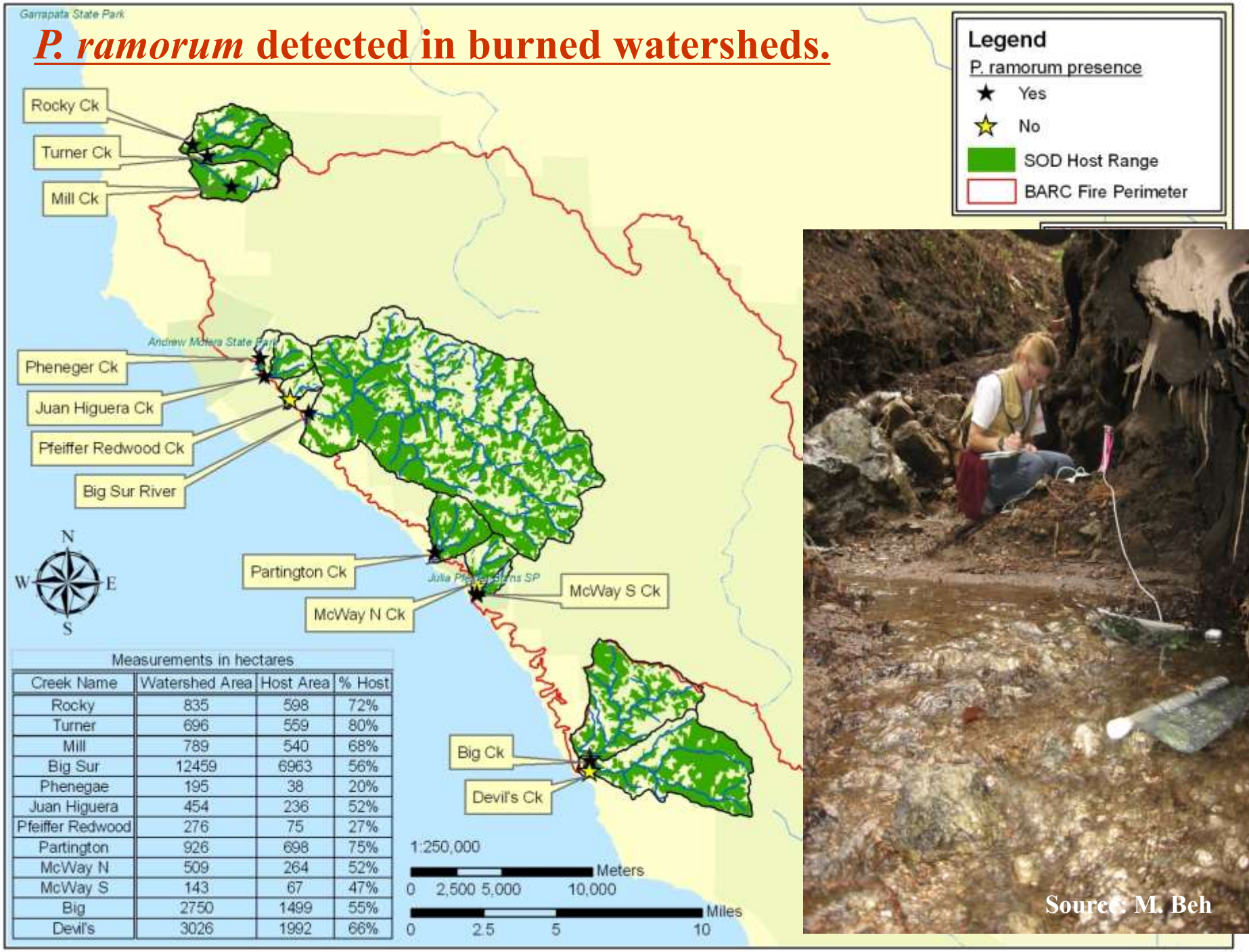
Image: K. Frangioso

SOD make fires worse?

Early stage → canopy scorching.

Late stage → soil damage.

P. ramorum detected in burned watersheds.



Measurements in hectares

Creek Name	Watershed Area	Host Area	% Host
Rocky	835	598	72%
Turner	696	559	80%
Mill	789	540	68%
Big Sur	12459	6963	56%
Phenegae	195	38	20%
Juan Higuera	454	236	52%
Pfeiffer Redwood	276	75	27%
Partington	926	698	75%
McWay N	509	264	52%
McWay S	143	67	47%
Big	2750	1499	55%
Devil's	3026	1992	66%

P. ramorum recovered from heavily burned forest:

from Soil

& Basal Sprouts of

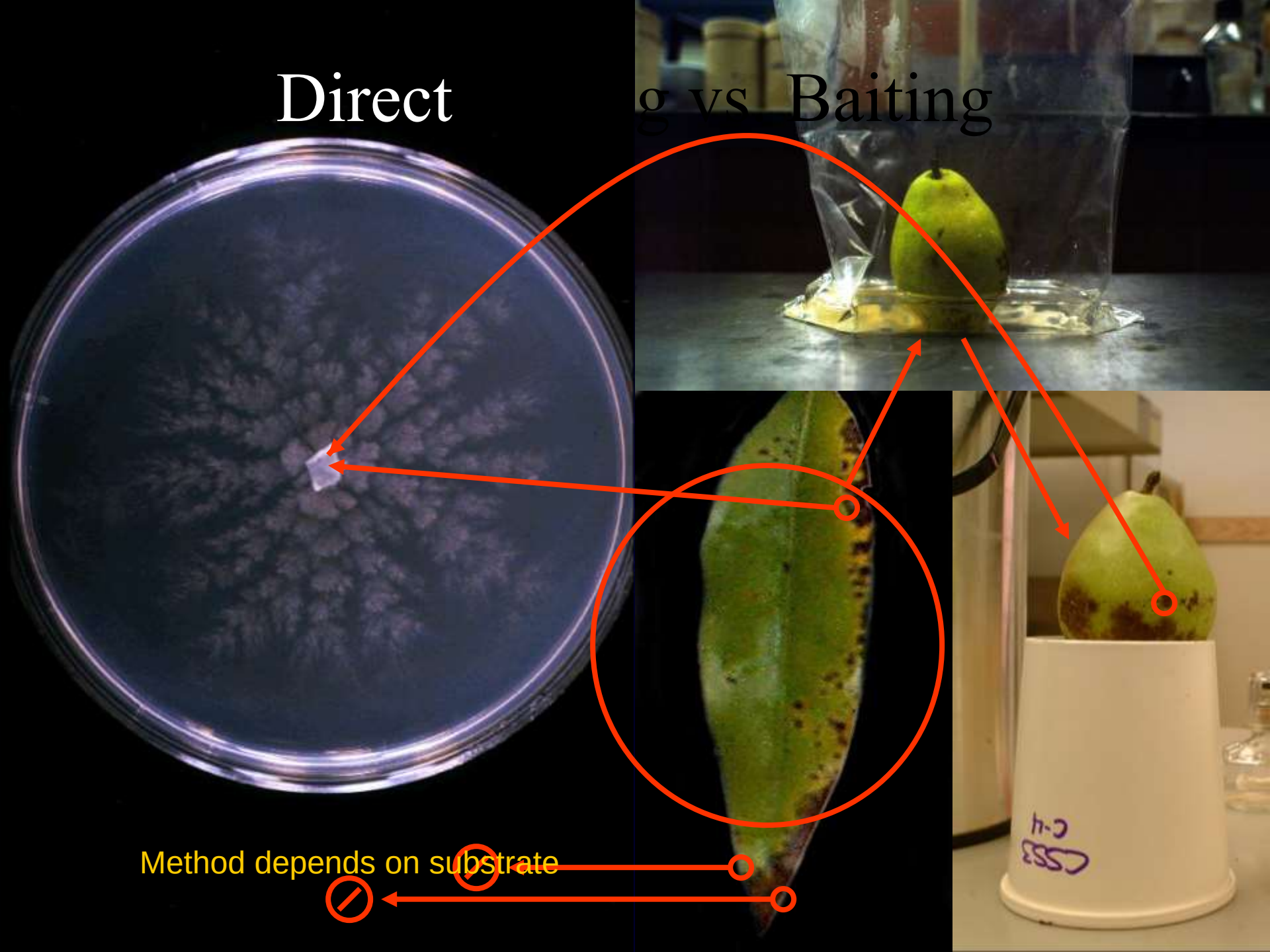
Tanoak & Bay Laurel

(all previously infested sites)

DIAGNOSTICS

- **Traditional isolation**, baiting, and morphological description
- **DNA-direct**: look for *P. ramorum* DNA itself
- **DNA-indirect:** proteins, elicitins
- **Quantitative PCR**: its applications and importance
- **Testing the process**, the procedure
- **Comparative testing** of reliability and sensitivity of different methods

Direct g vs. Baiting



Culturing, baiting, etc.

- **Knowledge is increasing** on tricks necessary to grow *P. ram* from plants and other substrates. Morphology is easy to recognize
- **False negatives** are frequent: mycelium is ephemeral, strong seasonality, huge effect of substrate (inhibitors), sensitivity to antibiotics, limited time window to isolate,

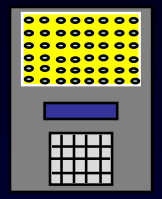
Overview of methods

collect symptomatic material

dry material, then grind

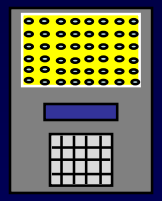
extract bulk DNA

1st round PCR reaction (using phyto1,4)

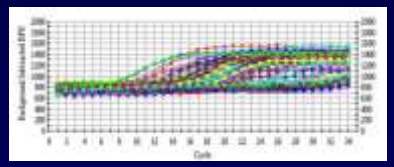


Amplify in thermocycler

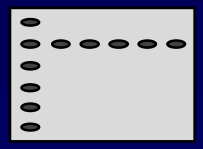
2nd round PCR reaction (using phyto2,3) with first round products (1:500 dilution) Opt. SYBR green



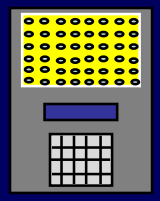
Amplify in light-cycler



light-cycler detects fluorescent products and determines melt temperatures



Electrophoresis of 2nd round PCR products



Amplify in thermocycler

Why molecular diagnostic

- Some microbes are hard or impossible to culture
- Morphology of microbes can be extremely variable (interpretation will be subjective)
- Cryptic species: sometimes associated with critical traits, e.g. host range

DNA-based diagnosis: major principles

- Diagnosis relies on one or a few loci, not on the whole genome

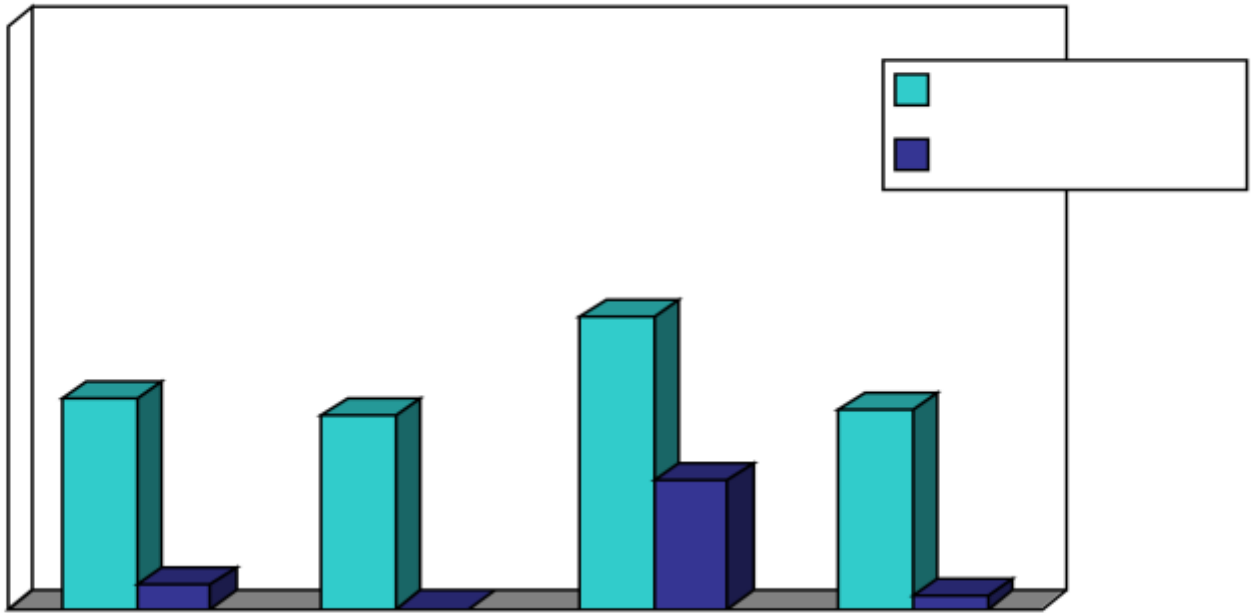
- **DNA sequence database (e.g. GENBANK) will determine potential *locus* of choice**
- ***Locus* needs to be informative at the right taxonomic level: e.g. <u>conserved</u> within species and <u>variable</u> between species**
- **Approach needs to capitalize on sequence differences among related species (probably a good idea to know nature of the sequence one is using): generally this is done by designing taxon-specific primers**
- **Method sensitivity will depend on nature of selected locus: single- vs. multiple loci, nuclear vs. mitochondrial**
- **Method needs to include variable independent ways to verify results in order to avoid false positives**

Two conceptually different approaches:

- DNA probes that will detect an entire group of pathogens
- As a second step, refine the diagnosis for a specific pathogen
- Good sensitivity, results will be very informative regarding a variety of microbes

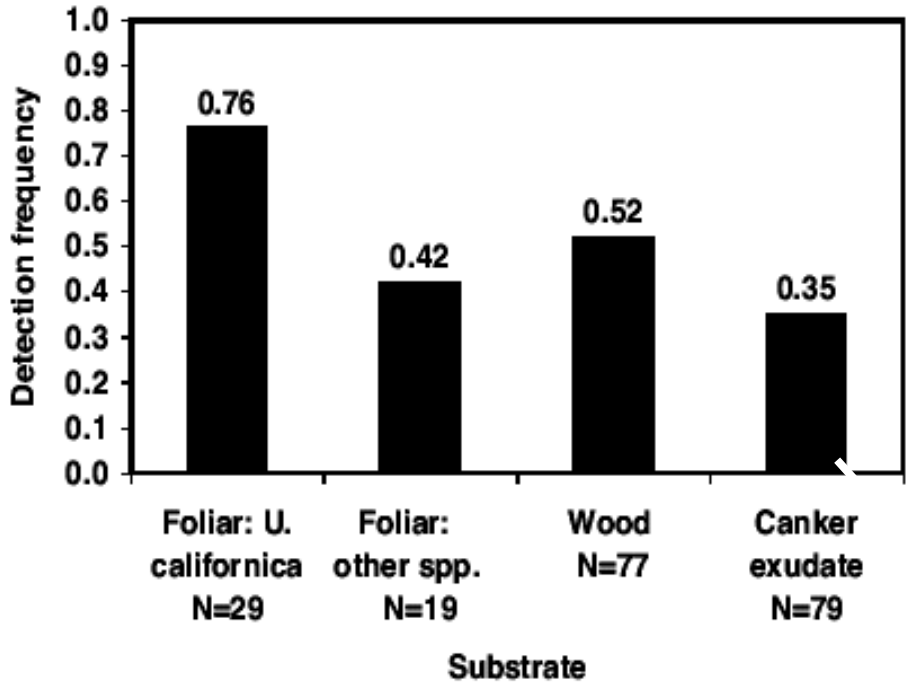
- DNA probe that are very specific for one pathogen
- Most sensitive technique, but obviously will only provide info one 1 microbe

PCR success is much higher than isolation suc
“difficult” samples.

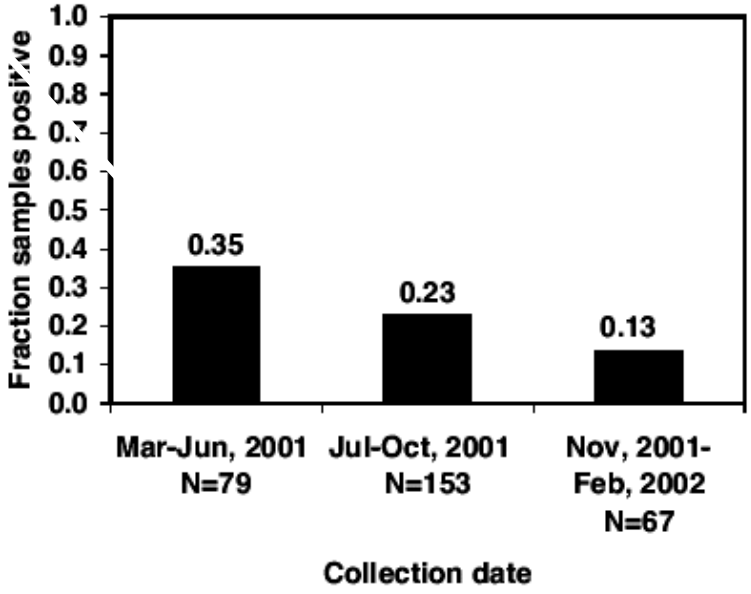


Significant effect of diagnostic type (P <0.001) and sample type (P=0.0036)

FIELD DATA (from Hayden et al. 2004)

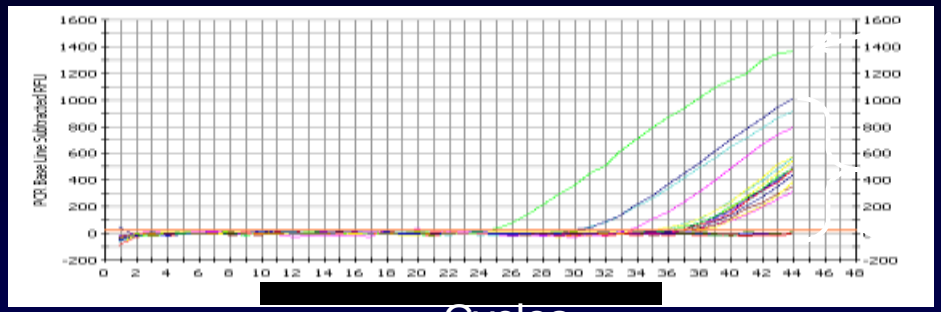


Effects of substrate and season →



Quantitative PCR

- Allows to check for specificity of PCR product
- Allows to quantify amount of target DNA
- Allows to quantify effects of different substrate/extraction

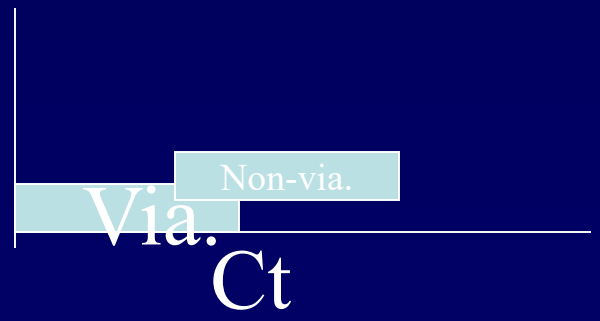


Cycles

More DNA---------Less DNA

Applications of Q-PCR

- **VIABLE vs. NON V.**
- Bay laurel leaves were separated in two groups based on success of isolations
- Mean Ct were significantly different and predicted distributions were not totally overlapping



- **ASSESS IF SENSITIVITY OF ASSAY WAS SUFFICIENT**
- Use PCR to determine whether *P. ram.* DNA present in finished compost
- PROBLEM: substrate strongly affects PCR results.
- Approach: amount of DNA detectable in all samples was determined and then used Q-PCR to find out whether sensitivity was sufficient

- Assay capable of detecting 240 g/ml ^10-5. Samples had at least one order of magnitude more DNA

Forest-Regional level

Avoid introduction, early detection, quarantine system is important as no real solution to problem exists once pathogen is introduced

P. ramorum is not *P. ramorum* is not *P. ramorum*
US lineage (A2) EU lineage (A1) New strain in WA
Avoid even regional movement of infected plant material is important

Daddy, everybody know
preventing new infestat
the best thing to do!!!!!

Moore Foundation

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SOD SQUAD