• We use the term lineage, to indicate clearly distinct (genetically and phenotypically) groups of strains within a species. How many lineages of *P. ramorum* are there in the US ornamental industry and how many are there in California?

• How do we explain the large distribution range of SOD in California?

• What was the change in US and international policy brought on by *P. ramorum*?

• Do you remember some of the ecological impacts caused by SOD?
P. ramorum growing in a Petri dish (2000)
*Phytophthora ramorum*

**Sporangia**

**Chlamydomospores**
SOD pathogen called *Phytophthora ramorum* arrived to California in 80s on infected ornamental plants.
Known distribution of *Phytophthora ramorum* in August 2000

Positive isolation

*P. ramorum*
*P. ramorum* introduced at least 12 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.
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

Yearly

Bay Coast Live Oak (no sporulation)

Canker margin in phloem

Wave years

Bleeding canker

Sporangia

Soil
Life Cycle of the West Nile Virus

**SUMMER**

Warm, wet weather produces large mosquito populations

Virus amplified among birds and mosquitoes

**SPRING**

Virus overwinters locally or is reintroduced

**FALL**

Mosquito populations decline, birds migrate

Dead-end hosts

Some birds die
Oaks
Coast live oak  
*Quercus agrifolia*
Black oak  
*Q. kelloggii*
Shreve’s oak  
*Q. parvula var. shrevei*
Canyon Live oak
Tanoak
*Notholithocarpus densiflorus*
Tanoak leaves also very infectious: tanoaks behave both as oaks and bay:

Tanoak infection does not require bay laurel
Hypoxylon (Anulohyphoxylon) fruitbodies on the main stem are a sign that a tree is functionally dead.
Only health compromised trees attract bark and ambrosia beetles: frass (sawdust) on the trunk is a sign of insect colonization
Tanoak vs. Oak mortality

- Tanoak – Big Sur
  - 70%

- Marin – predicted
  - 15 years for 90%
  
  All size classes
  Infection yearly if rain
  Leaves infectious
  Small number of sporangia necessary

- Oak – Big Sur
  - 40%

- Marin – predicted
  - 35 years for 90%
  
  Small trees not affected
  Infection only when Spring rainfall high
  Not infectious
<table>
<thead>
<tr>
<th>Confirmed Susceptible Species</th>
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<td>Andrew's clintonia bead lily</td>
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<td>Rosa species &amp; hybrids</td>
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Symptoms on Buckeye leaves and petioles
Scorching of maple leaves caused by *P. ramorum*
Primary cause of death is girdling of phloem, vessel blocking, while secondary organisms accelerate the process

Girdling occurs much faster than visible symptoms on crown. Girdled trees can survive apparently “green” 4 years + after being girdled

Girdled trees can fail even if “green” because of activity of secondary organisms

While insecticides may temporarily prolong the life of treated trees, beetle attacks are a good indicator of “hazard” trees
By inoculating with zoospores and without wounding, the ideal conditions for infection were figured out: these conditions are present in California especially when there are rainy late Springs: these conditions do not happen every year.
SOD spore catches in water: mid-April to mid-June is consistent.
Synchrony pathogen-host: 
Host susceptibility

Susceptibility of bay laurels 
(lesion size in nature)

Susceptibility of oaks 
(lesion size in lab)
When multiple rain events occur sporangia are produced on leaves of infectious hosts such as bay laurel and tanoak and can be airborne mostly up to 100 m but when winds are strong up to 2 miles.
New infections occur almost exclusively during the rainy season in or near sites previously infested

Limit tree-care and forestry work during wet season

If necessary to work in wet season, schedule to work in uninfested sites before working in infested sites

*P. ramorum* has a short spread range, knowing its fine scale distribution is essential
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
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
Effect of Bay Removal on SOD Spore Counts

Combined *P. ramorum* Spore Counts 2005 & 2006

Bay Laurels Removed

Untreated Control
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.

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

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.
In a survey of 2000 trees observed and isolated from 3 times a year for four years, we have determined less than 5% of bay laurels carry over infection after the 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
Injection Treatment
Preventive treatment that strengthens response of oaks: we developed an alternative to injection.
Topical Treatment
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
Transmission of SOD Through Pruning Tools

Infection (%)

Treatment

Positive Control
Untreated
Brush
Ethanol
Bleach
Lysol
Negative Control

2nd Exp 11/2010

Pruning Tools:
- Handsaw
- Chainsaw
- Control

2nd Exp 11/2010
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.
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.

The search for the Holy Grail of resistance:
10 sites, 229 trees

13,000

800
Common garden seedling tip assays of families indicates role of genetic variation within host species

![Graph showing asymptomatic mortality and median lesion/stem counts for different families 10 months post inoculation.](image-url)
Survival highest in families with at least average growth, and leaf or stem resistance.

Predicted survival of seedlings with and without selection based on resistance to *P. ramorum*
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
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
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 water samples from 2005 to the present.
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 Mettenmeyer; UNC Charlotte, Ted Swiecki, Phytophthora 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. Frangieso
SOD make fires worse?

Early stage → canopy scorching.
Late stage → soil damage.
P. ramorum detected in burned watersheds.
*P. ramorum* recovered from heavily burned forest:

from **Soil**

& **Basal Sprouts** of Tanoak & Bay Laurel

(all previously infested sites)

Photo: Heather Mehl
Combined effect of fire and SOD

• Mortality of medium sized redwoods

Effect of drought is significant reduction in populations of the pathogen on bay laurels (bay is reservoir of inoculum). Approximately only 5% carry infection: weak point in epidemiology
Final remarks

• SOD is an example of an environmental disaster due to poor regulation of a specific trade that is unrelated to forests

• In ornamental nurseries there are three distinct lineages of *P. ramorum*: with significant differences. Only 1 lineage is present in forests: it is essential to prevent further introductions in California

• Despite the limited dispersal ability the pathogen is solidly established mostly for two reasons: high infectivity and huge reproductive potential

• The pathogen is constrained by ecological factors: such as UV light, temperature and even related species that occupy the same niche (e.g bay laurel leaves)

• As of 2012, only 15% of the habitat favorable to *P. ramorum* has been occupied: we are only at the beginning of the epidemic. The example of Sudden Larch Death in the UK and Ireland shows the potential for unexpected outcomes
P. cinnamomii in Western Australia

- Causes disease sometimes referred to as “jarrah dieback”
- 1921 first jarrah deaths; 1964 deaths shown to be caused by P. cinnamomii.
- 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. cinnamomii distribution
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
Morphological phenotypes

- Sporangia
- Oospores
- Growth rates at different temperatures
- Significant differences even within a genotype
Typical *P. cinnamomomi* sporangia
Within a *P. cinnamomomi* lineage

There is substantial:

- Morphological diversity, and
- **PATHOGENIC DIVERSITY**

- We now may know why thanks to *P. ramorum* (have to come tomorrow)
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
• WHY? To reduce chances of species mixing and opportunities for HYBRIDIZATION
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. cinnamomomi* 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. cinnamomomi* (?)
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.
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 soil (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
Because of almost total susceptibility to soilborne *P. cinnamomoi*
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
Genetic structure of *P. cinnamomoni* tells us

- Same genotypes found in distant part of the world but on same crop. Evidence of efficient long distance movement

- Genotypes in the wild match genotypes in different trades, suggesting different trades/industry responsible for the infestation of different areas

- One new genotype is extremely aggressive; should regulation be imposed for new strains?
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
Predisposing conditions

• Serpentine soils, with limited microbial activity (hence outbreaks often in mining areas)

• Alternation of wet and dry periods i.e. Mediterranean climates: infection occurs in wet periods but disease advances during dry months

• Hard pan with underground water table
And in the greater SF Bay Area

- *P. cinnamomi* associated with root infections and tree decline of California Bay laurels and Pacific madrones. Also threatening another manzanita, the pallid manzanita

- Normally in association with human disturbance (roads, landscaping, urban development etc) including estates with lush gardens

- Interesting genetic homogeneity of strains, mostly linked to ornamental plant industry
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

Ornamental plants are everywhere
Once introduced, these organisms are almost impossible to eradicate.
Management options

- Avoid soil movement or movement of infected plants
- Eradicate most infectious hosts
- Use of composts: effect is both direct on pathogen, but more importantly indirect with plant roots
- Soil Fumigation and Solarization
- Phosphonates
Thousand canker disease of walnuts

*Geosmithia morbida*: fungus

*Pityophthorus juglandis*: insect vector
Some facts about TCD

- Insect is native
- Fungus apparently is not native
- Association between insect and fungus happened in the Southwest (1990s?)
- Disease started in Southwest, moved to California, from California elsewhere in the USA
- Insect of the genus *Pityophthorus* normally attack small sized portions of trees, not this one
- Mortality is due to coalescence of large numbers of cankers, and not to spread of a single lesion
- Black walnuts are the only hosts: *J. californica*, *J. hindsii*, and their hybrids with *J. nigra* (are all susceptible)
Walnut twig beetle

Small size of walnut twig beetle, Pityophthorus juglandis (photo by Eric Day).

The walnut twig beetle, Pityophthorus juglandis (photo by Eric Day).
Distribution of TCD as of 2009
Distribution of TCD as of 2013
Distribution of Jn in Eastern US
Hypotheses on spread

• The most likely pathway for movement is raw wood (logs, burls, stumps, firewood, wood packaging material (WPM)). Other potential pathways include nursery stock, scion wood for grafting, and natural spread. The beetle/pathogen complex is likely to enter the east with each entrance event, as follows:

- Movement of untreated walnut (logs, burls, stumps, firewood) across the country from the west into eastern states appears limited but it does occur and it is rarely documented. Low grade walnut maybe utilized if bark is attached this could be an important pathway. Raw wood is the most critical pathway.

- Campsites and sawmills in the Great Plains states may facilitate the eastern movement of Thousand Canker Disease.

- To date there have been no reports of infected trees in walnut production nurseries; however, if nurseries do become infected, this could become an important pathway.

- Natural spread along riparian corridors is likely to occur.
Symptoms from a distance

- Early = flagging
  Late = dead canopy with
Close-up symptoms

Pinhead sized entry hole of beetle
Close-up symptoms

Larvae tunnels produced along the grain, and so do the lesions.
Close-up symptoms

Mortality caused by coalescence of multiple cankers.
Colonies of Gm on both sides
Fungus sporulating on beetle and in beetle gallery
WTB is believed to have 2 to 3 generations a year in California. Adults emerge for an initial flight period in April and May followed by a longer second generation flight period in mid-July to mid-September. After flying, male beetles initiate brood galleries on branches often near leaf scars or lenticels.

Males produce a pheromone and attract 2 to 3 females, which attract additional beetles to the tree. Females deposit eggs in galleries (tunnels) that are directed against the grain and constructed in the phloem and xylem (wood) surfaces. The gallery imprint is left on the wood surface. Small white C-shaped larvae hatch and create feeding mines that extend from the egg galleries. These mines are contained in the phloem and filled with dark brown to black-colored boring dust.

Larvae complete development in the mines and subsequently pupate within a single pupal cell. Adults emerge and either remain at the original tree or fly to other trees to mate and reproduce. WTB does not appear to be attracted to stressed or injured branches or trees.

Beetles are believed to inoculate the *Geosmithia* sp. fungus into the phloem during construction of feeding or reproductive galleries. The fungal pathogen colonizes and kills the phloem. Dead tissue is limited to the phloem and cambium and the fungus does not penetrate woody tissues. Secondary saprophytic fungi may opportunistically colonize the wood beneath cankers.
Control?

- Bark application and drenches with insecticides apparently not effective
- Prevention is the best option
- Some efficacy reported with injections of insecticides, fungicides, and fertilizers
• What are the differences between aerial and soil-borne *Phytophthora* species?

• Reconstructing the genetic relationship of different strains of *P. cinnamommi* has helped us learn at least two important things. What are they?

• What are the three cases of *P. cinnamommi* outbreaks in California?

• How does 1000 canker disease spread and how can one control it?