California invaded: 1849 A.D.

Port Orford Cedar Root Disease
1950s

Sudden Oak Death
1990s

Pitch canker disease
1980s

Colored canker of sycamore 70s

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

Dutch Elm Disease
1960s

White pine blister rust
1930s

Oak root canker
2000

Manzanita die-back 2004

Xylella scorch of maples 2000s

1000 canker disease of walnuts
2001

Expansion of root pathogens
Post 1880s
Oomycota

- Belong to a kingdom that includes kelp and diatoms
- 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 not 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 interrupted 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 uniforms.
Phytophthora

• Some important plant pathogens, with very well known history
  – *Phytophthora infestans* and the Irish potato famine
  – *Phytophthora 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 behardier
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. hyberinalis*, *P. ramorum*

• **Extreme temperatures**: ability to withstand extreme cold or heat. Normally depends on resting structures, and where they are produced.
  
  Species producing abundant chlamydomspores 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. cinnamomii*
• *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 transmission 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 cinnamomomi

- 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
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. cinnamomomi* 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 \textit{in planta}

• Controls many \textit{Phytophthora} diseases
Effectiveness of Phosphite

- Phosphite contains, but does not stop colonization by *P. cinnamomii* 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. cinnamommi* (?)
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
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 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
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. cinnamommi* come from?

- Avocado orchards once surrounded oak woodlands infected by *P. cinnamommi*
- 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
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
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
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

Hosts

Environment

Interactions

Man
Starting in 1994 in Santa Cruz County (but probably arrived late 1980s)
2013 SOD damage, Brookings, 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)
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
*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.
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 20°C; 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)

Bay

Sporangia

Wave years

Canker margin in phloem

Soil

Bleeding canker

Yearly

Wave years
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
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.

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
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

Matteo’s Lab

Viet Cong
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

2\textsuperscript{nd} Exp 11/2010

Pruning Tools:
- Yellow: Handsaw
- Blue: Chainsaw
- Green: Control

Infection (%)

Treatment
- Positive Control
- Untreated
- Brush
- Ethanol
- Bleach
- Lysol
- Negative Control

Pruning tools investigated include:
- Handsaw
- Chainsaw
- Control

2\textsuperscript{nd} 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
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.

10 mos post inoculation

- **Asymptomatic**
- **Mortality**
- **Median lesion / stem**

<table>
<thead>
<tr>
<th>Family</th>
<th>Asymptomatic</th>
<th>Mortality</th>
<th>Median lesion / stem</th>
</tr>
</thead>
</table>
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
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, with a focus on detecting and mapping the spread of diseases that threaten forest ecosystems.
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 $\rightarrow$ canopy scorching.

Late stage $\rightarrow$ 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
DIAGNOSTICS

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

Method depends on substrate
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

1. Collect symptomatic material
2. Dry material, then grind
3. Extract bulk DNA
4. 1st round PCR reaction (using phyto1,4)
5. Amplify in thermocycler
6. Electrophoresis of 2nd round PCR products
7. Light-cycler detects fluorescent products and determines melt temperatures
8. 2nd round PCR reaction (using phyto2,3) with first round products (1:500 dilution)
10. Amplify in light-cycler
11. 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. conserved within species and variable 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 success on "difficult" samples.

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

FIELD DATA (from Hayden et al. 2004)
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

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.
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 knows preventing new infestations is the best thing to do!!!!!!