Phytophthora: A guide to molecular analyses

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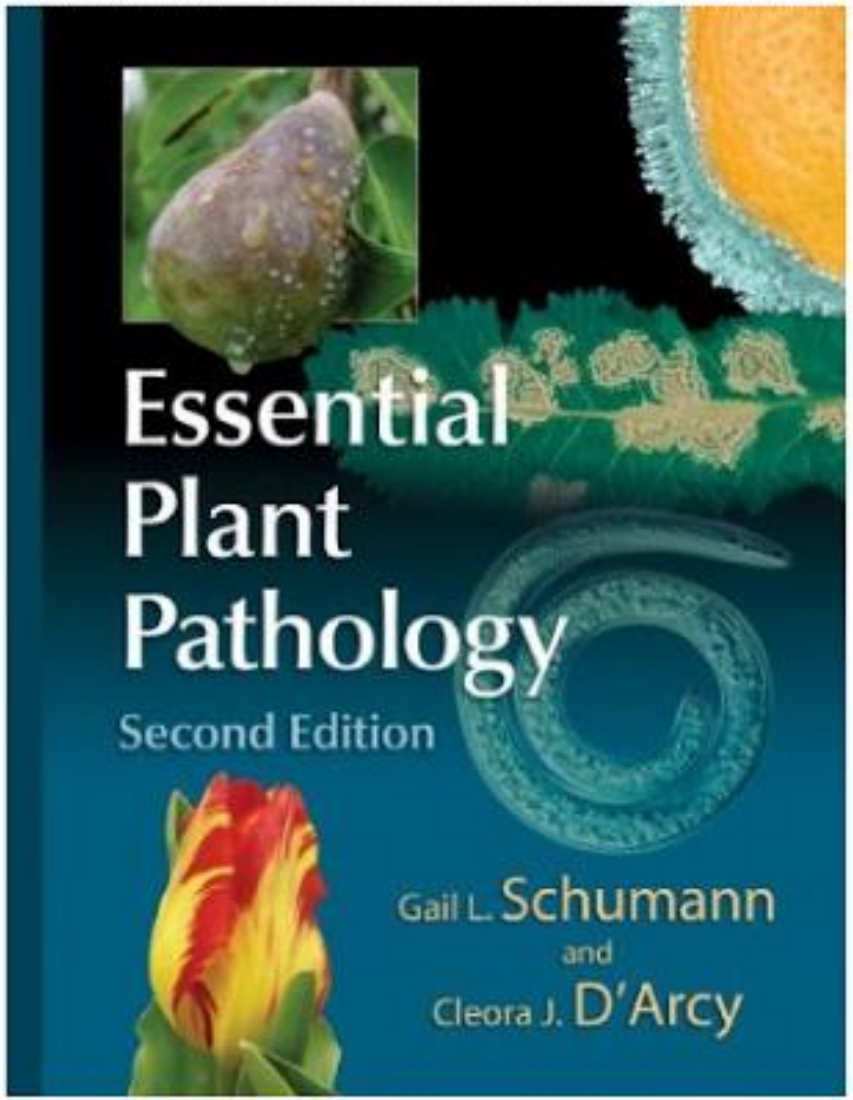
Horticulture & Crop Science
Cal Poly, San Luis Obispo

Circa late 2002…

Cal Poly Strawberry Center, 2016



***Phytophthora* species** (*phyto* = **plant,** *phthora* = **destroyer)**

- **Nonseptate** hyphae
- **Sexual spores:** oospores; paragynous or amphigynous antheridium; usually only one antheridium per oogonium
- **Asexual spores:** zoospores produced by typically lemon-shaped sporangia
- **Diseases:** stem, crown, and root rots; diseases of lower tissues reached by water splashing up from the soil

Phytophthora infestans causes late blight of potato and tomato, the disease that led to the Irish potato famine in the 1840s and the beginning of the science of plant pathology.

Phytophthora ramorum causes the devastating ramorum blight (sudden oak death) on the U.S. west coast and was probably introduced into the United States on rhododendron nursery stock.

Phytophthora cinnamomi is a widespread, destructive root and crown pathogen throughout the tropical world.

Phytophthora… an old enemy

Dozens of species detected in coastal California on:

- avocado
- asparagus
- cauliflower (rare)
- citrus
- grape
- pepper
- raspberry
- sage
- spinach (rare)
- strawberry
- tomato
- numerous ornamentals
- and forest plants

Phytophthora in ornamentals

Hundreds of ornamental plants are susceptible.

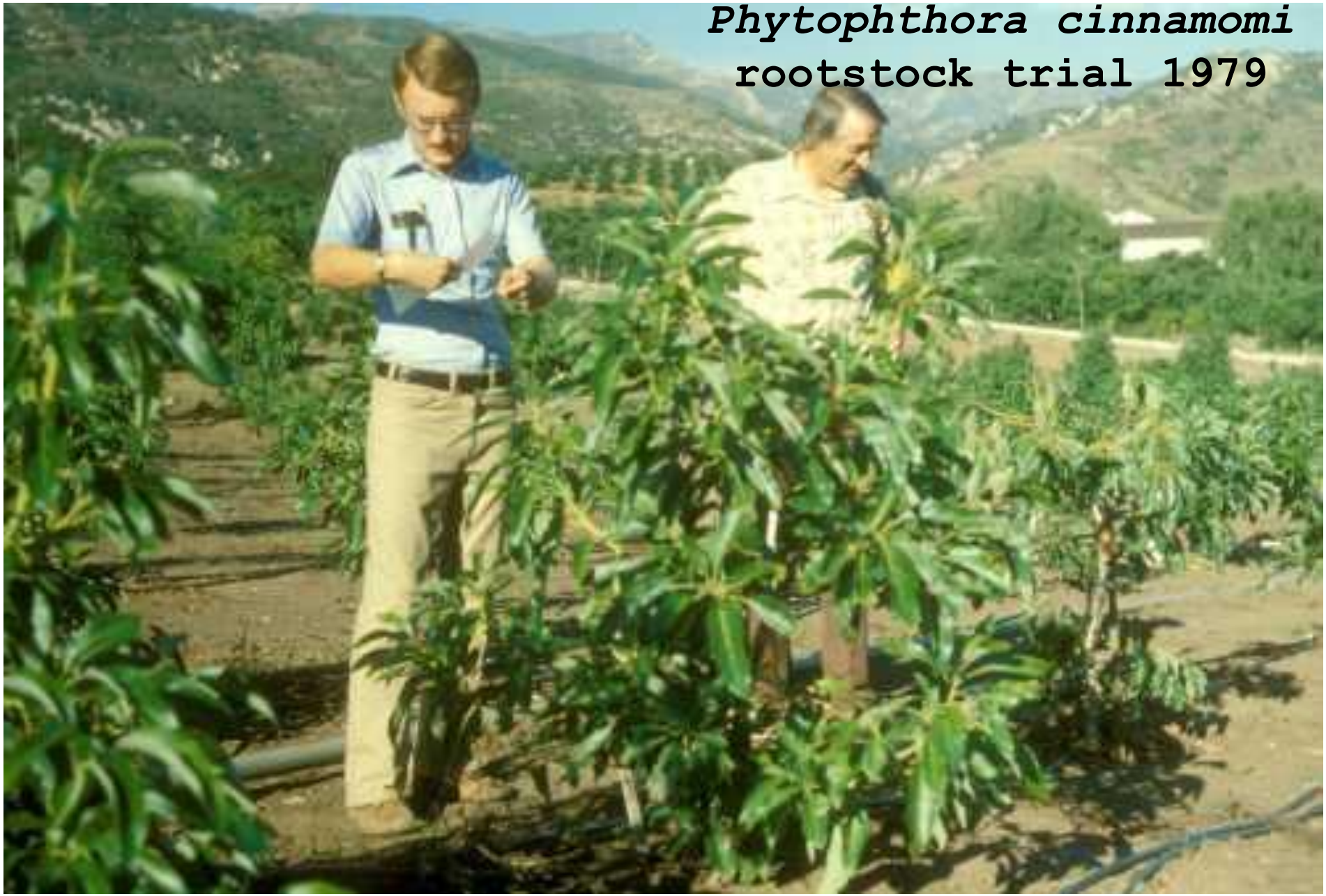
Incite root rot, crown rot, and foliar blights.

Caused by a few dozen *Phytophthora* species in U.S.

***cinnamomi, cryptogea, citricola,citrophthora, cactorum, cambivora, drecshleri, foliorum, gonapodyides, heveae, hibernalis, nicotianae, palmivora, ramorum, syringae, tropicalis…* plus many more.**




Phytophthora… an old enemy

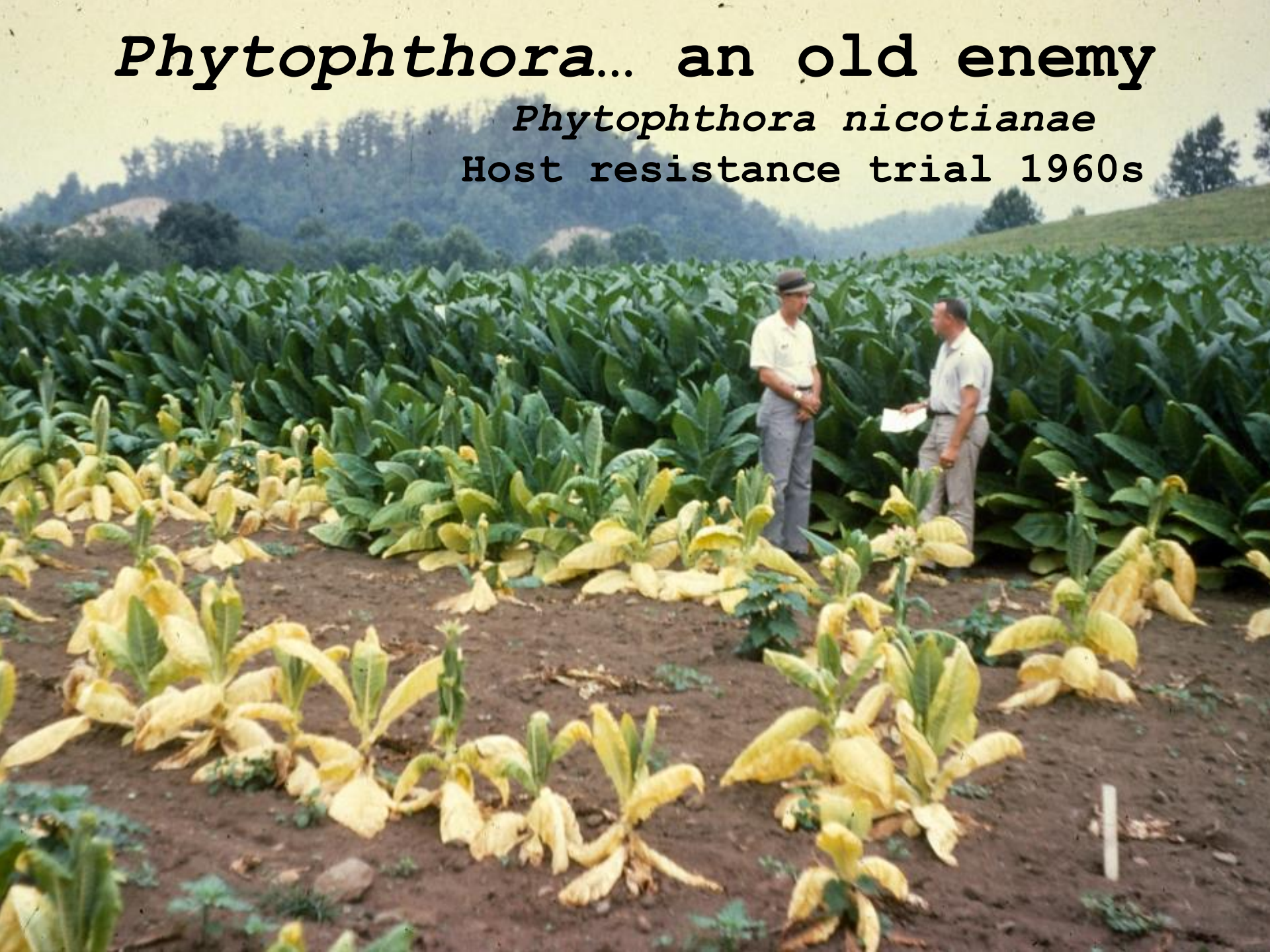


Phytophthora… an old enemy

Phytophthora… an old enemy

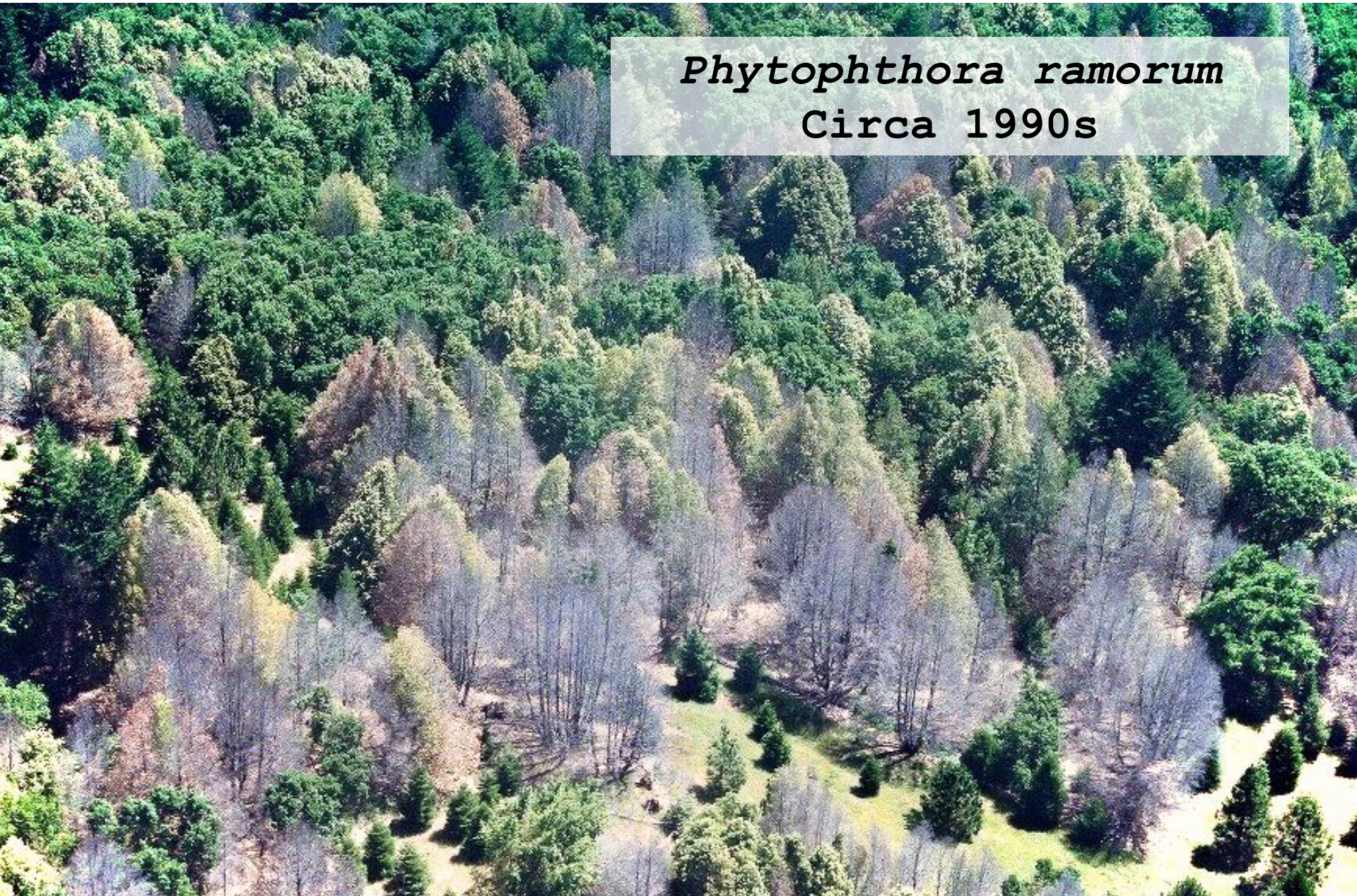
Phytophthora nicotianae

Host resistance trial 1960s



Phytophthora… an old enemy

Phytophthora… a new enemy



Phytophthora… a new enemy

Phytophthora… a new enemy

Photo: Phytosphere Research

Pest Alert

Phytophthora tentaculata

Phytophthora tentaculata has been detected in several California native plant nurseries and restoration sites. These are the first detections of *P. tentaculata* in the USA. *Phytophthora tentaculata* was initially noticed in a native plant nursery causing a severe root and crown rot in sticky monkey flower, *Diplacus aurantiacus* subsp. *aurantiacus* (Scrophulariaceae) in 2012 (figure 1). Since then it has been detected in four additional nurseries in three counties in CA in addition to three restoration sites where outplanted stock was found to be infected.

The more you look, the more you find…

Extensive surveys have been conducted in historically underexplored ecosystems to determine the spread of invasive species in forest decline worldwide

New records in 2007 collected by PDIC

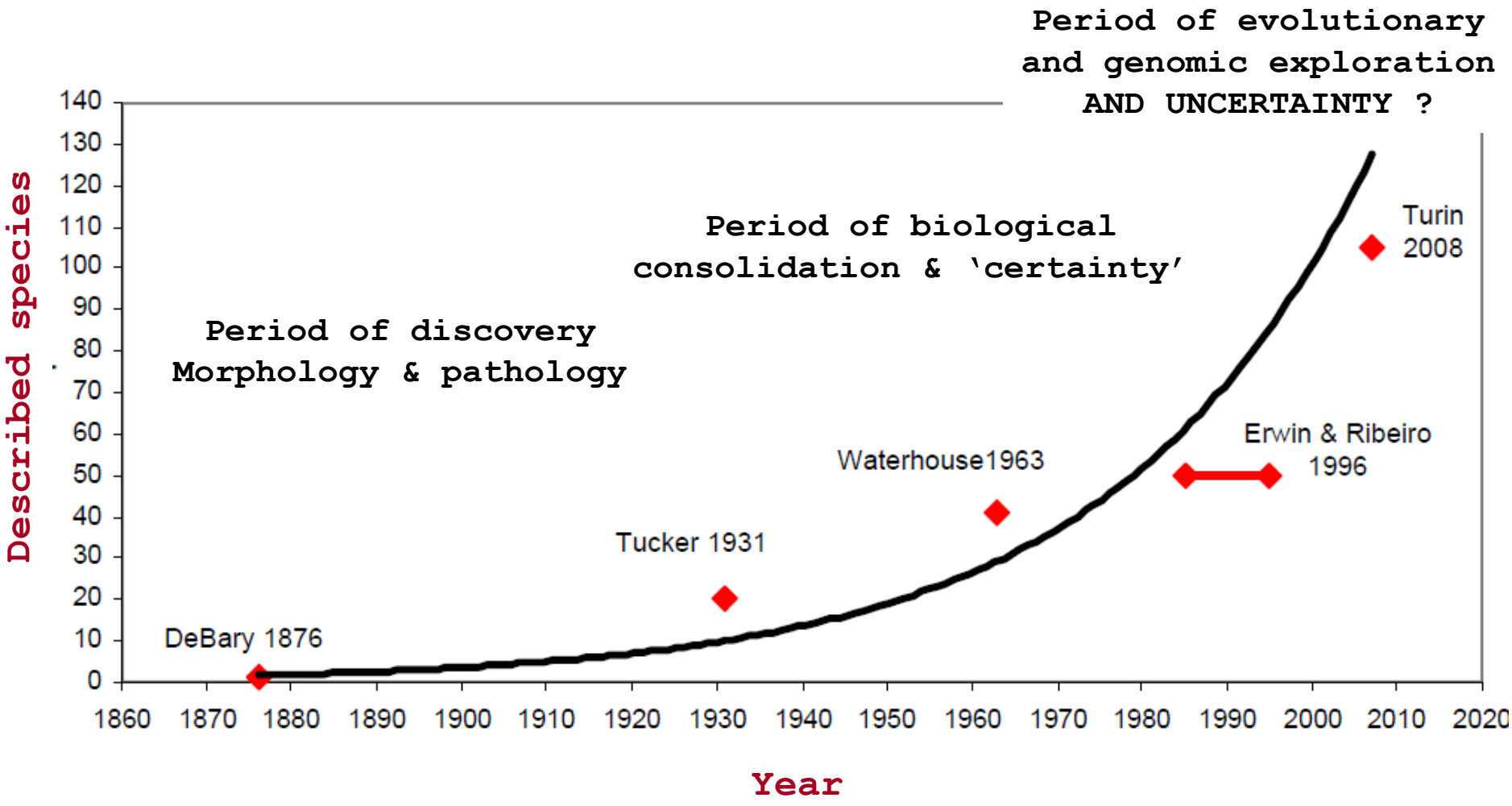
Host	Common Name	Fungus	Record
Itea virginica	Sweetspire	*Phytophthora cinnamomi*	US
Loropetalum chinense		*Phytophthora cinnamomi*	US
Hemerocallis sp.	Daylily	*Phytophthora nicotianae*	NC
Nerium oleander (leafspot)	Oleander	*Phytophthora palmivora*	US
Plumbago auriculata	Leadwort	*Phytophthora nicotianae*	US
Ilex crenata	Japanese Holly	*Botryosphaeria rhodina*	NC
Buxus sempervirens cv. Suffruticosa	English Boxwood	*Phytophthora palmivora*	US
Cyclamen persicum		*Phytophthora tropicalis*	US
Liriodendron tulipifera	Tuliptree	*Phytophthora inundata*	US
Euphorbia maculata	Spotted Spurge	*Phytophthora dreschleri*	US
Vinca minor	Lesser Periwinkle	*Phytophthora palmivora*	US
Juniperis Scopulorum	Rocky Mountain Juniper	*Seiridium cupressi*	US
Juniperis Scopulorum	Rocky Mountain Juniper	*Seiridium unicorne*	NC
Hydrangea macrophylla		*Corynespora cassiicola*	NC
Leucothoe sp.	Dog hobble etc.	*Phytophthora nicotianae*	US
Leucothoe sp.	Dog hobble etc.	*Glomerella cingulata*	US
Edgeworthia sp.	Paperbush	*Phytophthora nicotianae*	US
Eriobotrya japonica	Loquat	*Phytophthora cactorum*	NC
Lagerstroemia Indica	Crapemyrtle	*Phytophthora palmivora*	US
Hedera Helix	English Ivy	*Phytophthora palmivora*	NC

The more you look,
the more you find…

Phytophthora Populations in Nursery Irrigation Water in Relationship to Pathogenicity and Infection Frequency of *Rhododendron* and *Pieris*

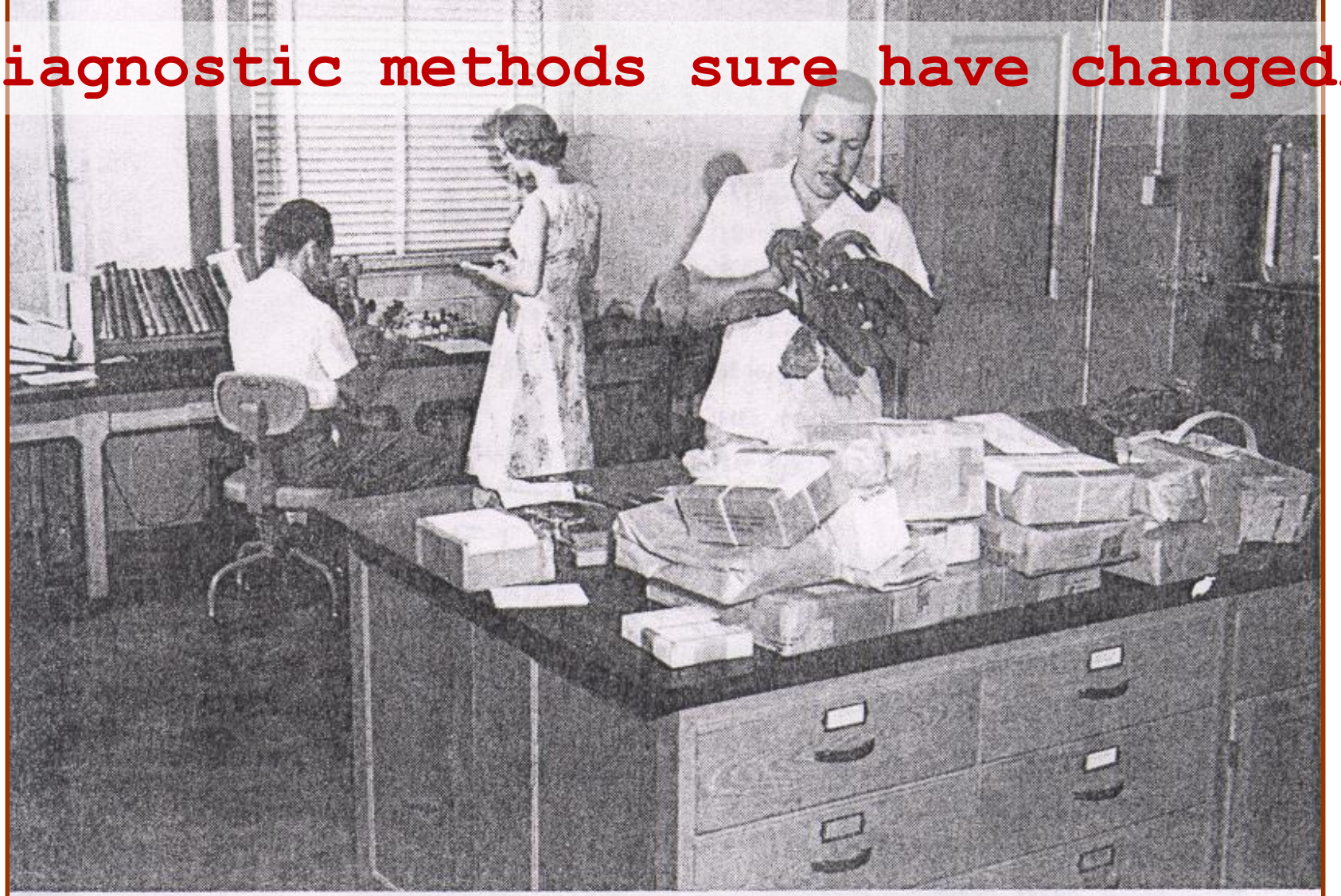
A. L. Loyd, D. M. Benson, and **K. L. Ivors,** Department of Plant Pathology, North Carolina State University, Raleigh 27607

Described *Phytophthora* species over time



Slide from C. Brasier

Diagnostic methods sure have changed…



PLANT DISEASE CLINIC - ESTABLISHED 1951

Fig. 22. Attacking the "morning mail" in the plant disease clinic ca. 1959. Faculty member J. L. Apple, secretary, and Graduate Assistant John L. Ruehle

Plant Disease 2012:



Identification and Detection of *Phytophthora*: Reviewing Our Progress, Identifying Our Needs

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Department of Plant Pathology, NC State University, Mountain Hort. Crops Research & Extension Center, Mills River, NC



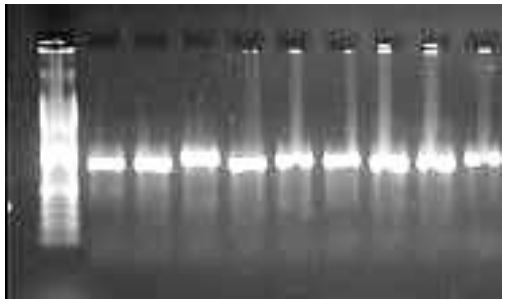
Phytophthora Diagnostics

- Isolation & culture morphology

Phytophthora Diagnostics

- Isolation & culture morphology

- Nucleic acid based approaches (PCR)



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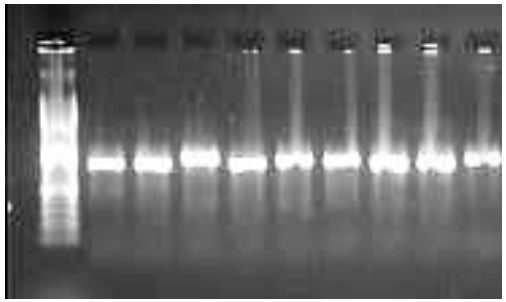

- Immunodetection (aka ELISA)
  - NOT species specific

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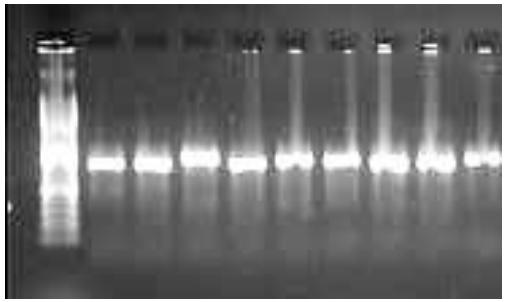

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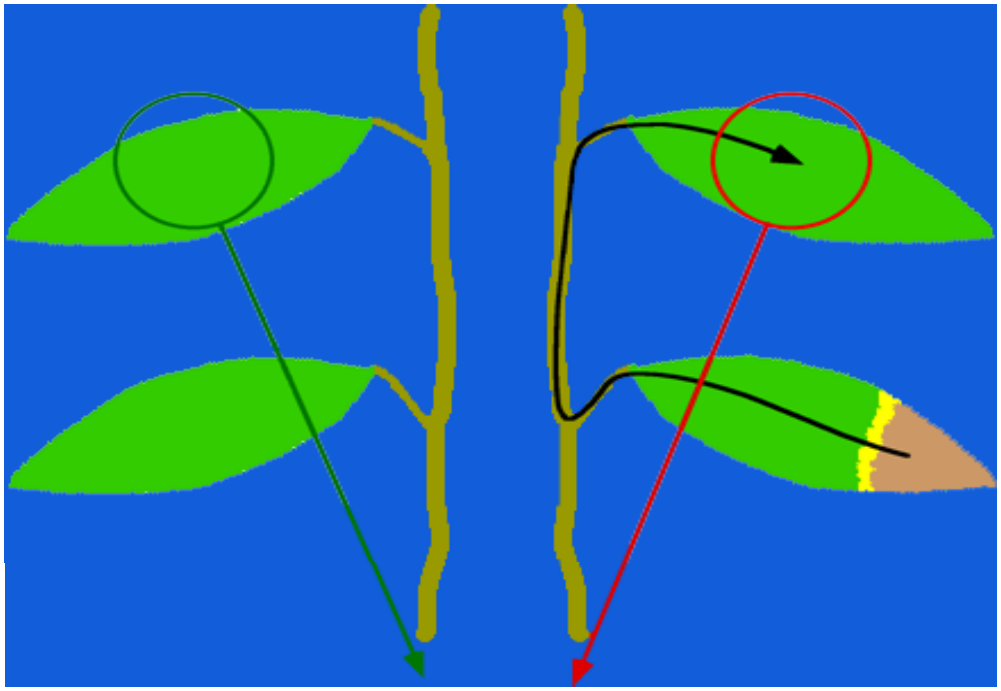
Phytophthora Diagnostics:
Immunodetection

Compare metabolite or protein profile from healthy vs infected plants



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Immunodetection

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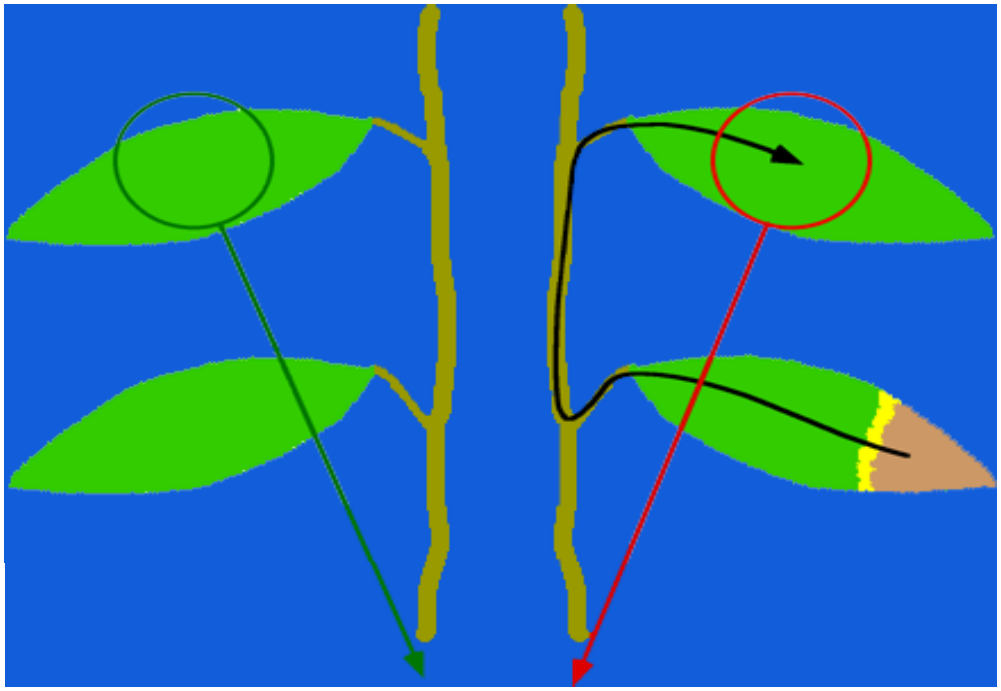
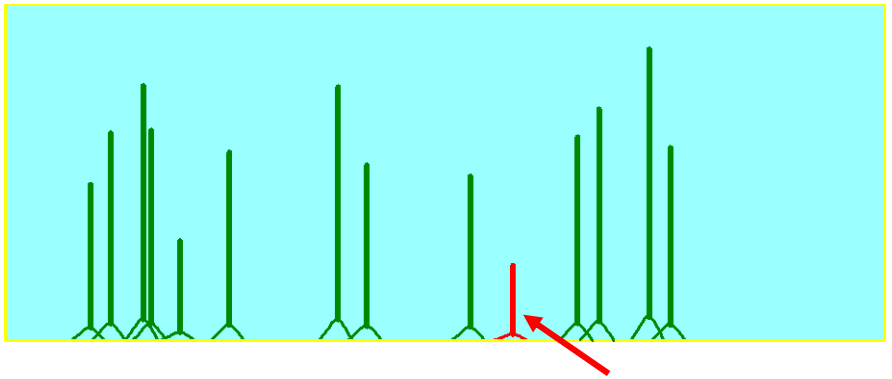


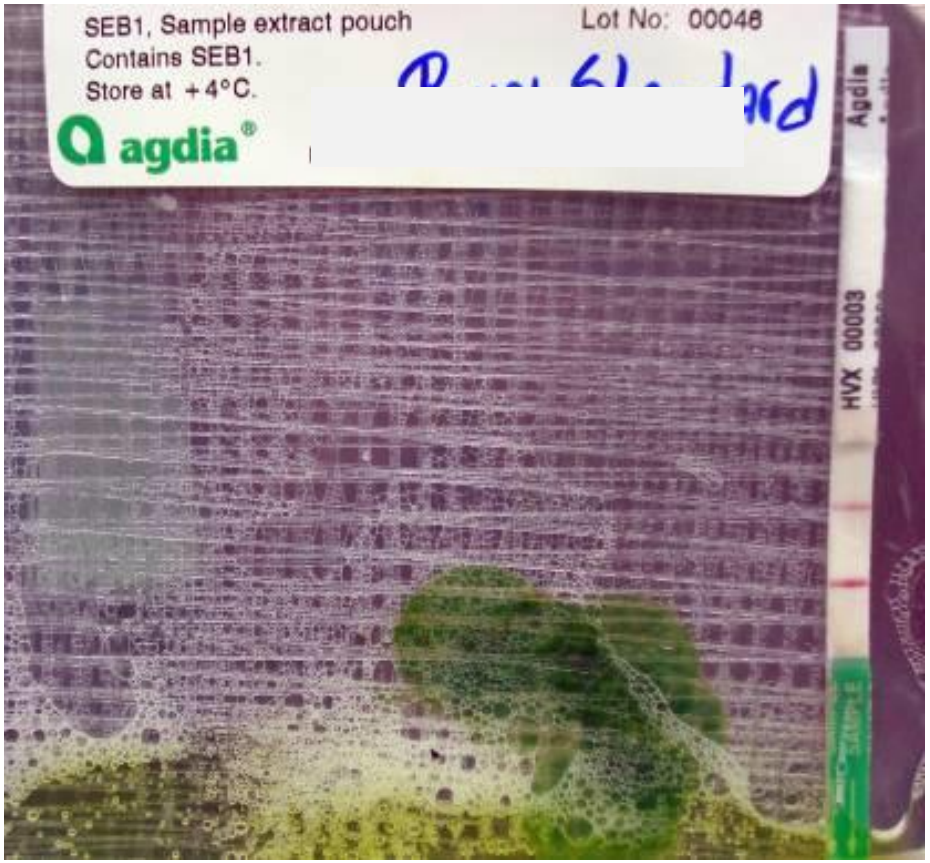
Develop antibody test for specific pathogen protein or metabolite

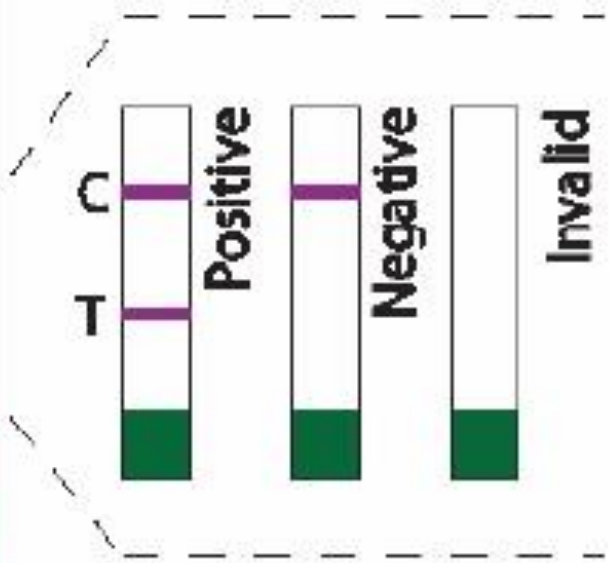


Phytophthora ImmunoStrip

agdia®

Leading the way to healthy crops.





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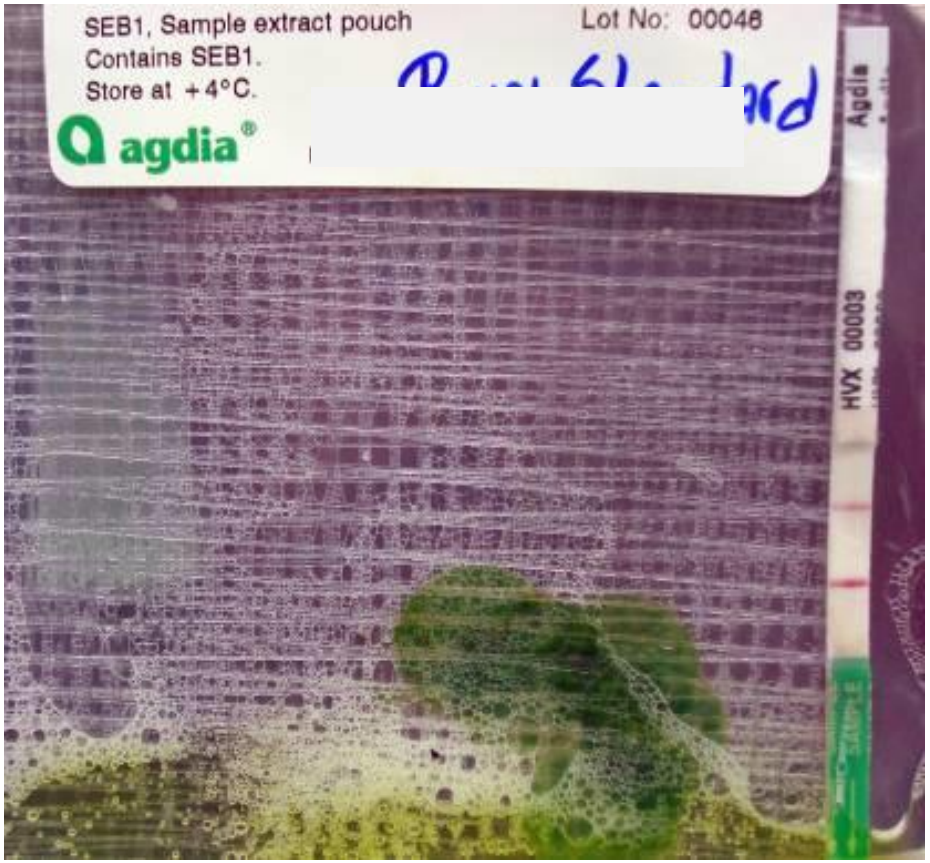
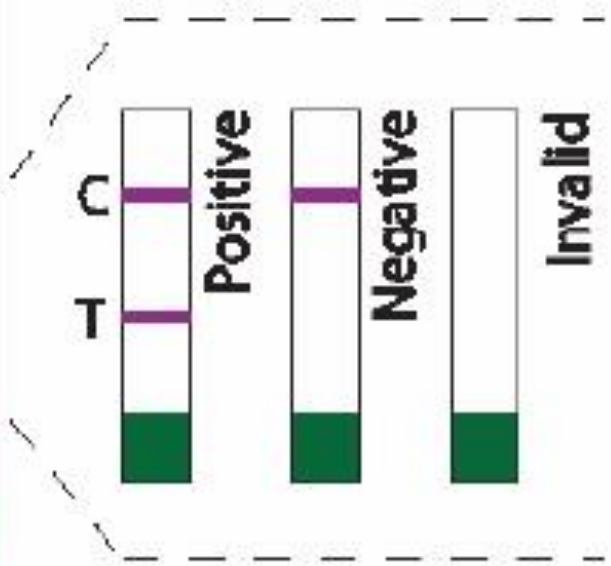
5 min; $5.00





Phytophthora ImmunoStrip

Osterbauer, N., and Trippe, A. 2005. Comparing diagnostic protocols for *Phytophthora ramorum* in rhododendron leaves. Online. Plant Health Progress.

from an Oregon nursery.

Leaf no.	DAS-ELISA*	Culture plating**	Nested PCR**
1	+	+	–
2	+	+	–
3	+	+	–
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	–
9	+	–	–
10	–	–	–
11	+	+	–
12	+	+	+
13	+	+	+
14	+	+	+
15	+	+	+
16	–	–	+
17	–	–	+
18	+	–	+
19	+	+	+
20	+	+	+
21	+	+	+
22	+	–	+
23	+	+	+
24	+	–	+

* Detects all *Phytophthora* species present.
** Species-specific diagnostic protocol for *P. ramorum*.

Osterbauer, N., and Trippe, A. 2005. Comparing diagnostic protocols for *Phytophthora ramorum* in rhododendron leaves. Online. Plant Health Progress.

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1	+	+	–
2	+	+	–
3	+	+	–
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	–
9	+	–	–
10	–	–	–
11	+	+	–
12	+	+	+
13	+	+	+
14	+	+	+
15	+	+	+
16	–	–	+
17	–	–	+
18	+	–	+
19	+	+	+
20	+	+	+
21	+	+	+
22	+	–	+
23	+	+	+
24	+	–	+

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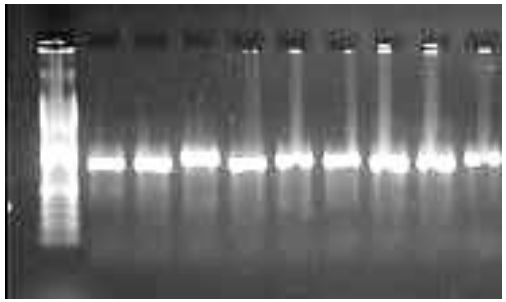
Phytophthora Diagnostics

- Isolation & culture morphology

- Nucleic acid based approaches (PCR)



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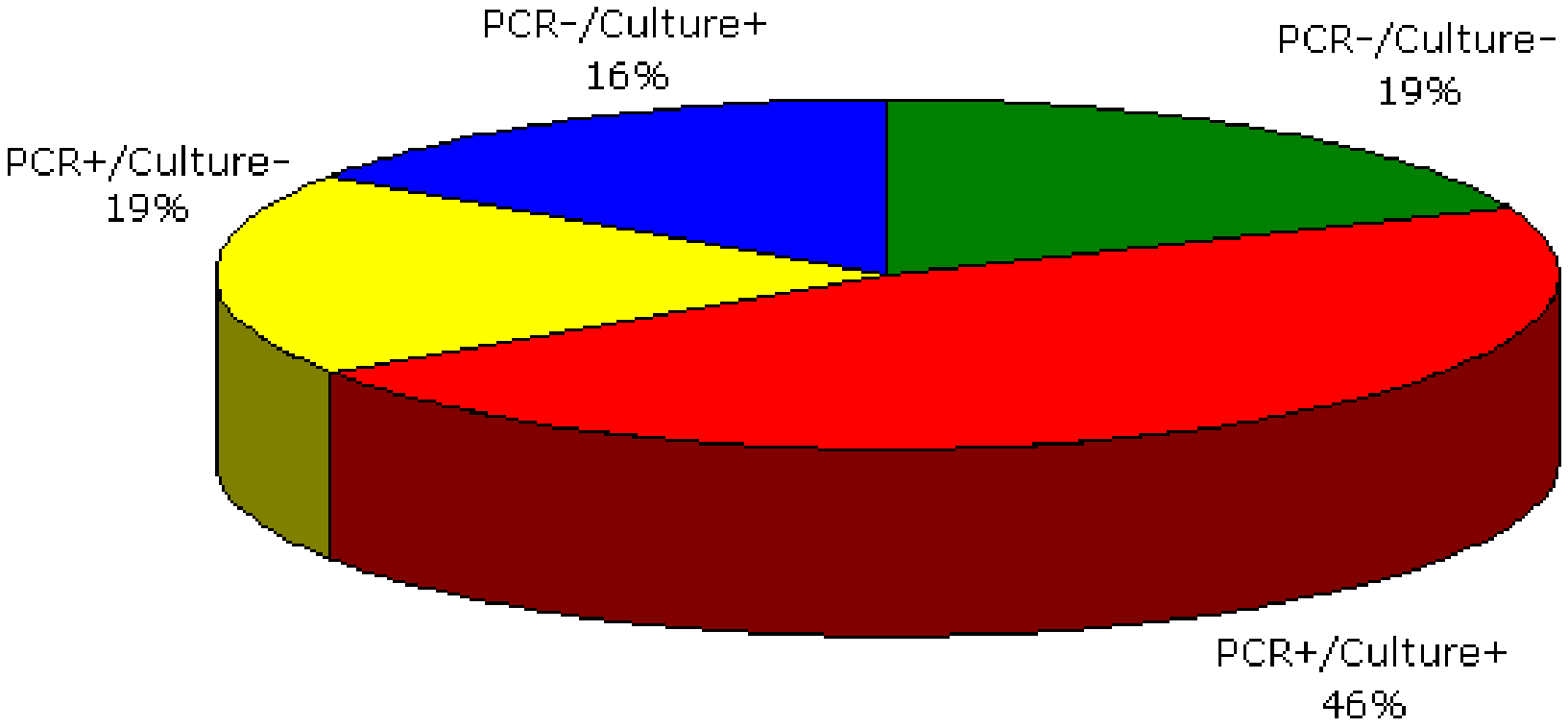


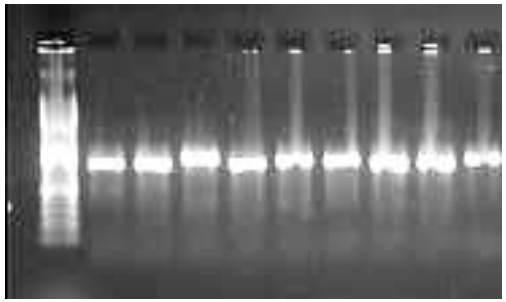
Combined results for the plant samples collected and tested for *Phytophthora ramorum* using the species-specific USDA-validated diagnostic protocols nested PCR (PCR) and culture plating (culture).

Osterbauer, N., and Trippe, A. 2005. Comparing diagnostic protocols for *Phytophthora ramorum* in rhododendron leaves. Online. Plant Health Progress.

Phytophthora Diagnostics

- Isolation & culture morphology

- Nucleic acid based approaches (PCR)



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Molecular Diagnostics

- Detect
- Quantify
- Identify
- Characterize population
  - determine strain or lineage

Molecular Diagnostics

- Detect (*in planta* / environmental samples)
- Quantify
- Identify
- Characterize population
    - determine strain or lineage

Molecular Diagnostics

To answer these questions…

Is Phytophthora species X present on the leaves?

Molecular Diagnostics

To answer these questions…

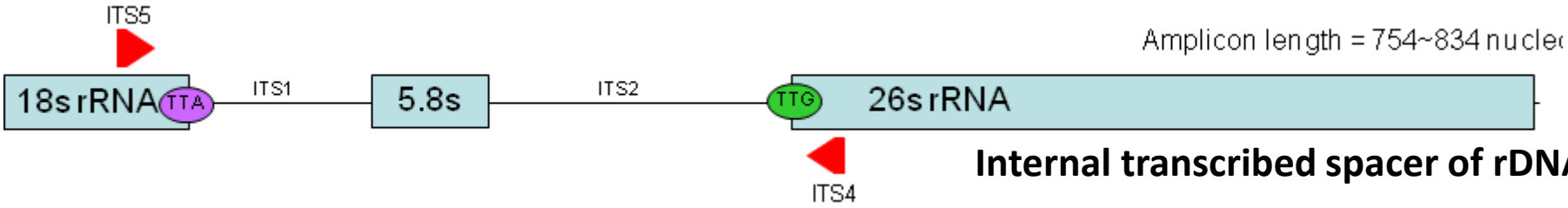
Is Phytophthora species X present on the leaves?

Use species specific DNA primers or probe to detect specific DNA in the plant sample

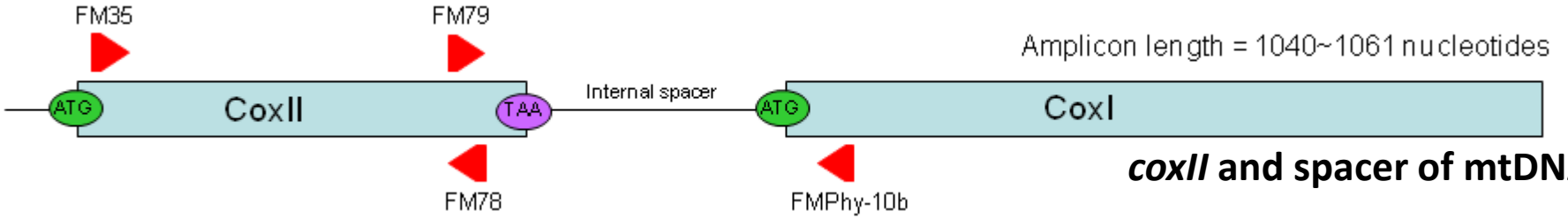
Molecular Detection
Primer / probe selection

- Look for a single region that is conserved within a species but variable between species
- Have conserved sequences flanking variable region

Loci used for Molecular Detection



Internal transcribed spacer of rDNA



***coxII* and spacer of mtDNA**

www.phytophthoradb.org

Molecular Detection
Primer / probe selection

- Look for a single region that is conserved within a species but variable between species
- Have conserved sequences flanking variable region
- Amplicon size suitable for real time PCR
- High copy number

Plant Disease 2012:



Identification and Detection of *Phytophthora*: Reviewing Our Progress, Identifying Our Needs

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Table 2. Selected references for species-specific diagnostic markers for detection of various *Phytophthora* spp.[a]

Species	Assay type	Locus	Reference
Phytophthora alni	Conventional	SCAR	Bakonyi et al. (16), De Merlier et al. (66), Ioos et al. (135)
P. boehmeriae	Conventional	ITS	Shen et al. (248)
P. cactorum	Conventional	SCAR	Causin et al. (52), Lilja et al. (181)
	Conventional	ITS	Bhat and Browne (25), Boersma et al. (33), Lacourt et al. (
	Real time	*Ypt1*	Schena et al. (239)
P. cambivora	Conventional	SCAR	Schubert et al. (245)
		ITS	Boersma et al. (33)
	Real time	*Ypt1*	Schena et al. (239)
P. capsici	Conventional	ITS	Silvar et al. (250), Ristiano et al. (231), Zhang et al. (299)
	Real time	ITS	Silvar et al. (249), Pavón et al. (224)
P. cinnamomi	Capture probe	*Cina*	Coelho et al. (56)
	Conventional	ITS	Boersma et al. (33), Williams et al. (293)
		Lpv	Kong et al. (162)
		SCAR	O'Brien (216)
	Real time	*Ypt1*	Schena et al. (239)
P. citricola	Conventional	ITS	Schuburt et al. (245)
	Real time	ITS	Böhm et al. (34)
		Ypt1	Schena et al. (239,240)
P. citrophthora	Conventional	SCAR	Ersek et al. (81), Goodwin et al. (97)
	Real time	ITS	Ippolito et al. (137), Schena et al. (241)
P. colocasiae	Conventional	ITS	Mishra et al. (207)
P. cryptogea	Conventional	*Ypt1*	Minerdi et al. (205)
		ITS	Boersma et al. (33)
	Real time	*Ypt1*	Schena et al. (239)
P. erythroseptica	Conventional	ITS	Nanayakkara et al. (210), Tooley et al. (267)
	Real time	ITS	Cullen et al. (63)
		rpb1	Atallah and Stevenson (13)
P. europea	Real time	*Ypt1*	Schena et al. (239)
P. fragariae	Conventional	ITS	Bonants et al. (35)
		ITS	Lacourt et al. (168)
		SCAR	Ioos et al. (135)
		ras	Ioos et al. (136)
		trp1	Ioos et al. (136)
	Real time	ITS	Bonants et al. (38)
P. fragariae rubi	Conventional	ITS	Schlenzig (242,243)
P. ilicis	Real time	*Ypt1*	Schena et al. (239)
P. infestans	LCR[b]	ITS	Tooley et al. (270)
	Conventional	ITS	Hussain et al. (129), Tooley et al. (267,269), Trout et al. (2
		SCAR	Judelson and Tooley (146)
	Real time	*ras*	Atallah and Stevenson (13)

Table 2. (*continued from preceding page*)

Species	Assay type	Locus	Reference
P. inundata	Real time	*Ypt1*	Schena et al. (239)
P. kernoviae	Real time	*Ypt1*	Schena et al. (239,240)
		ITS	Hughes et al. (125)
P. lateralis	Conventional	ITS	Winton and Hansen (295)
	Real time	*Ypt1*	Schena et al. (239)
P. medicaginis	Conventional	ITS	Liew et al. (180)
P. megasperma	Real time	*Ypt1*	Schena et al. (239)
P. melonis	Conventional	ITS	Wang et al. (284)
	Real-time	ITS	Wang et al. (284)
P. nemorosa	Real time	*Ypt1*	Schena et al. (239)
	Conventional	*cox* spacer	Martin et al. (198)
P. nicotianae	Conventional	SCAR	Ersek et al. (81), Goodwin et al. (96)
		elicitin	Lacourt and Duncan (169)
		ITS	Boersma et al. (33), Grote et al. (104), Huang et al. (123), Tooley et al. (267)
		parA1	Kong et al. (160)
		Ypt1	Meng and Wang (203)
	Real time	ITS	Huang et al. (123), Ippolito et al. (137), Schena et al. (241)
P. pinifolia	Conventional	ITS	Durán et al. (76)
	Conventional	*Ypt1*	Durán et al. (76)
P. pseudosyringae	Real time	*Ypt1*	Schena et al. (239)
	Conventional	*cox* spacer	Martin et al. (198)
	Real-time	*cox* spacer	Tooley et al. (271)
P. psychrophila	Real time	*Ypt1*	Schena et al. (239)
P. quercina	Conventional	SCAR	Nechwatal et al. (211), Schubert et al. (245)
	Real time	*Ypt1*	Schena et al. (239,240)
P. ramorum	Conventional	ITS	Garbelotto et al. (89)
		cox spacer	Martin et al. (198)
		SCAR	Ioos et al. (135)
		gpa1	Ioos et al. (136)
		trp1	Ioos et al. (136)
		cox1	Kroon et al. (167)
	Real-time	ITS	Bilodeau et al. (31), Hayden et al. (114,115), Hughes et al. (126), Tomlinson et al. (
		cox spacer	Tooley et al. (271)
		Ypt1	Schena et al. (239,240)
		elicitin	Bilodeau et al. (31)
		β-tubulin	Bilodeau et al. (31)
	LAMP[b]	ITS	Tomlinson et al. (264)
P. sojae	Real-time	ITS	Bienapfl et al. (27), Wang et al. (285)
Multiple spp.	PCR-ELISA	ITS	Bailey et al. (15)
Genus-specific	Conventional	ITS	Drenth et al. (71)
	Primers	*cox* spacer	Martin et al. (198)
	Real time	ITS[c]	Kox et al. (164)
		Ypt1	Schena et al. (239)

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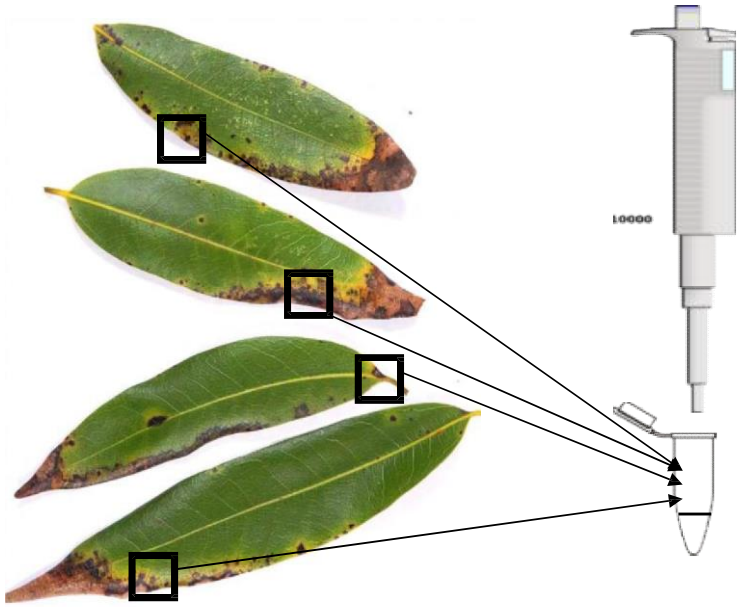
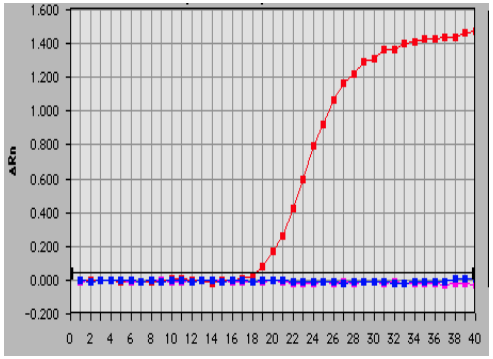
		ITS	Boersma et al. (33), Grote et al. (104), Huang et al. (123), Tooley et al. (267)
		parA1	Kong et al. (160)
		Ypt1	Meng and Wang (203)
	Real time	ITS	Huang et al. (123), Ippolito et al. (137), Schena et al. (241)
P. pinifolia	Conventional	ITS	Durán et al. (76)
	Conventional	*Ypt1*	Durán et al. (76)
P. pseudosyringae	Real time	*Ypt1*	Schena et al. (239)
	Conventional	*cox* spacer	Martin et al. (198)
	Real-time	*cox* spacer	Tooley et al. (271)
P. psychrophila	Real time	*Ypt1*	Schena et al. (239)
P. quercina	Conventional	SCAR	Nechwatal et al. (211), Schubert et al. (245)
	Real time	*Ypt1*	Schena et al. (239,240)
P. ramorum	Conventional	ITS	Garbelotto et al. (89)
		cox spacer	Martin et al. (198)
		SCAR	Ioos et al. (135)
		gpa1	Ioos et al. (136)
		trp1	Ioos et al. (136)
		cox1	Kroon et al. (167)
	Real-time	ITS	Bilodeau et al. (31), Hayden et al. (114,115), Hughes et al. (126), Tomlinson et al. (
		cox spacer	Tooley et al. (271)
		Ypt1	Schena et al. (239,240)
		elicitin	Bilodeau et al. (31)
		β-tubulin	Bilodeau et al. (31)
	LAMP[b]	ITS	Tomlinson et al. (264)
P. sojae	Real-time	ITS	Bienapfl et al. (27), Wang et al. (285)
Multiple spp.	PCR-ELISA	ITS	Bailey et al. (15)
Genus-specific	Conventional	ITS	Drenth et al. (71)
	Primers	*cox* spacer	Martin et al. (198)
	Real time	ITS[c]	Kox et al. (164)
		Ypt1	Schena et al. (239)

Phytophthora ramorum

P. ramorum	Conventional	ITS	Garbelotto et al. (89)
		cox spacer	Martin et al. (198)
		SCAR	Ioos et al. (135)
		gpa1	Ioos et al. (136)
		trp1	Ioos et al. (136)
		cox1	Kroon et al. (167)
	Real-time	ITS	Bilodeau et al. (31), Hayden et al. (114,115)
		cox spacer	Tooley et al. (271)
		Ypt1	Schena et al. (239,240)
		elicitin	Bilodeau et al. (31)
		β-tubulin	Bilodeau et al. (31)

Molecular Detection





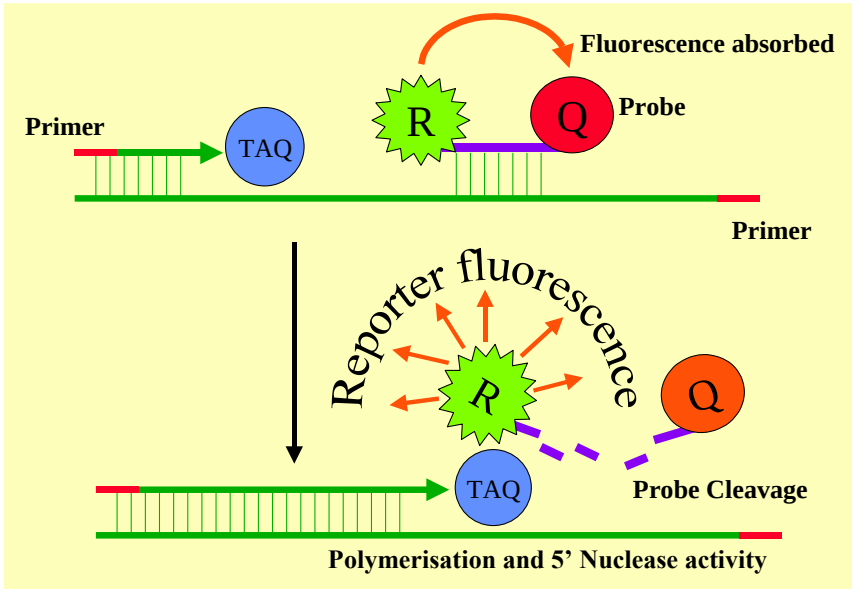
Sample leaf symptoms → Extract DNA → Amplify DNA with *P. ramorum* specific primers → Analyze the results!

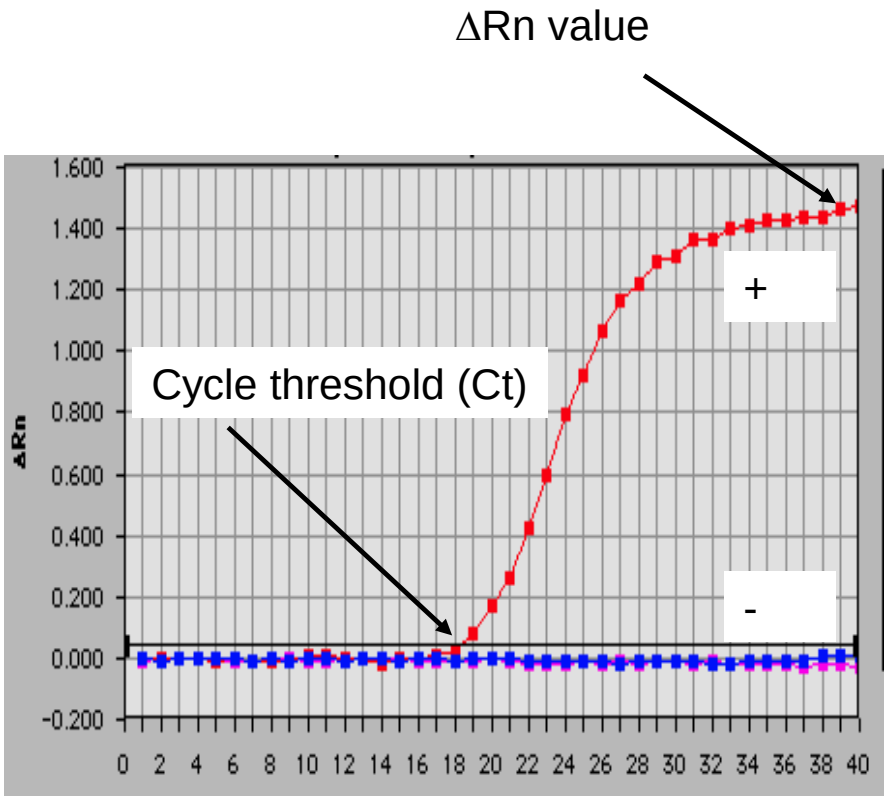
YES/NO

Molecular Diagnostics

- Detect (*in planta* / environmental samples)
- Quantify (*in planta* / environmental samples)
- Identify
- Characterize population
    - determine strain or lineage

Molecular Detection and Quantification

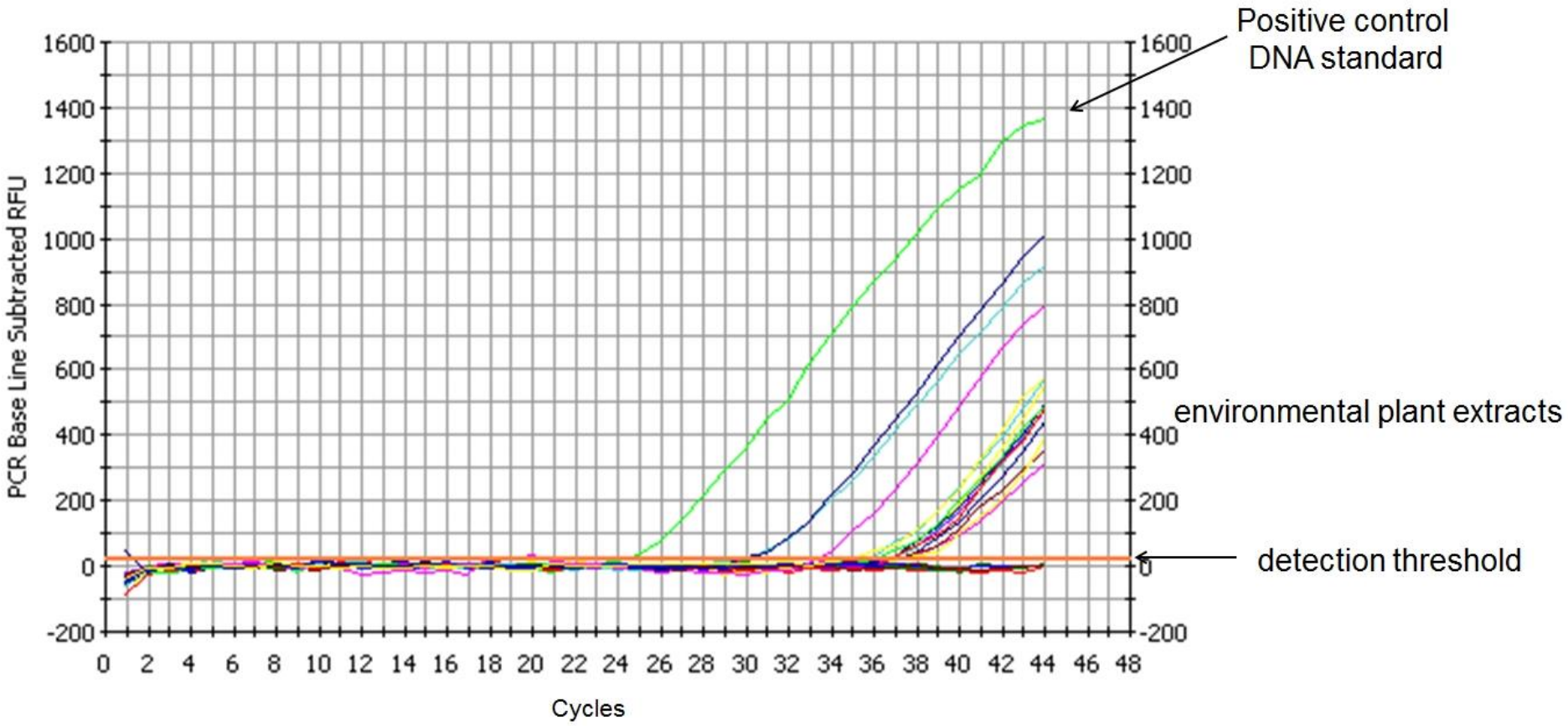




- Species specific probe labelled with fluorophore
- Florescence monitored real time
- Positive when Ct < 40

Real-time, TaqMan, QPCR

Molecular Detection and Quantification



Real time, TaqMan, QPCR

Molecular Detection
Technique to use?

Conventional vs Real-time PCR

- Impacts sensitivity and time to achieve results

Different chemistries for Real-time PCR

- TaqMan
- Molecular Beacons
- Scorpion

Single round PCR, Nested PCR, Real-time, TaqMan, QPCR?

Molecular Diagnostics

To answer these questions…

Is Phytophthora species X present on the leaves?

Use species specific DNA primers or probe to detect specific DNA in the plant sample

What species of Phytophthora is infecting the leaves?

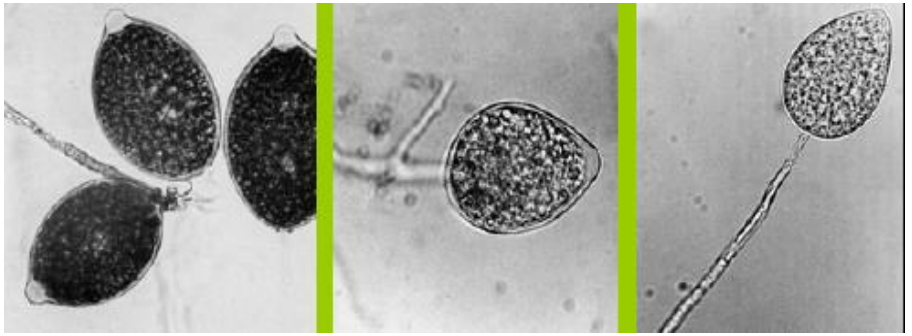
- Sequence a variable gene and compare it with other known isolates
- Build a tree and see which of the other sequences the unknown groups with – i.e. which is it most similar to

Molecular Diagnostics

- Detect (*in planta* / environmental samples)
- Quantify (*in planta* / environmental samples)
- **Identify (*in vitro* / isolates)**
- **Characterize population**
    - **determine strain or lineage**

Molecular Identification

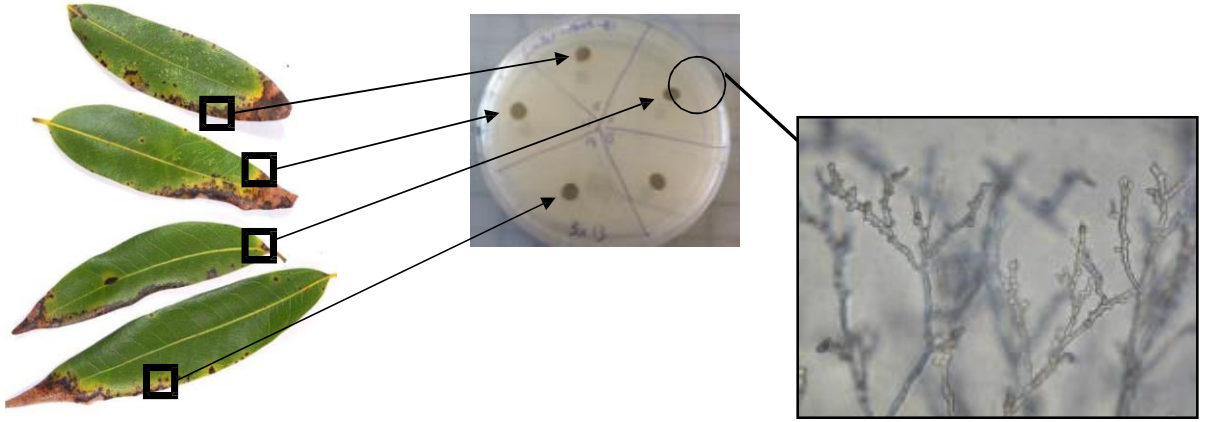
- Often takes less time
- Less subjective than morphology

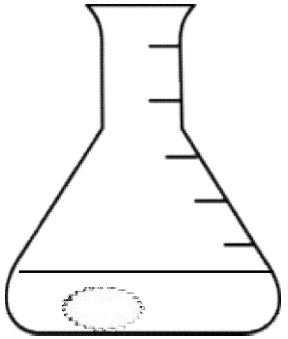


- Sometimes differentiates pathogen below species level (e.g. lineage)

Molecular Identification

DNA sequencing

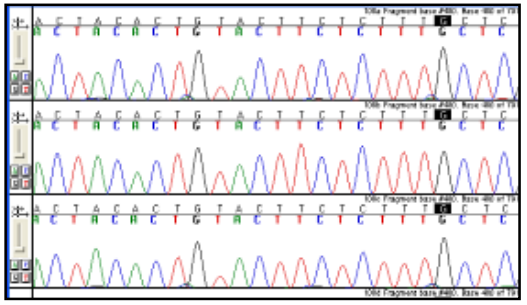


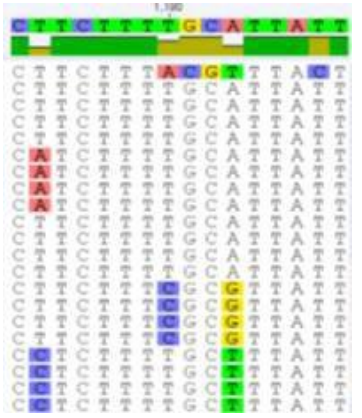


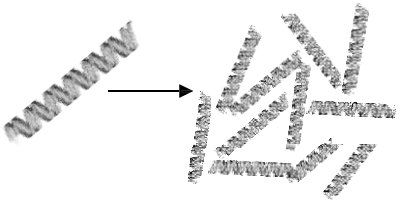
Sample leaf symptoms → Plate onto selective growth medium → Pure culture → Grow mycelium in liquid culture →

Molecular Identification

DNA sequencing





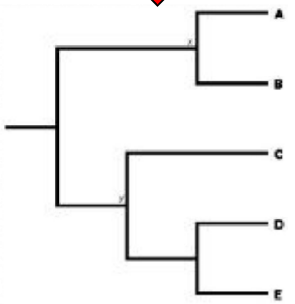


Extract DNA

Amplify specific region of DNA

Sequence DNA

Align with other known DNA sequences



Build phylogenetic tree

Molecular Identification

DNA sequencing

- Determine the exact sequence of As, Ts, Gs and Cs in a particular region of DNA

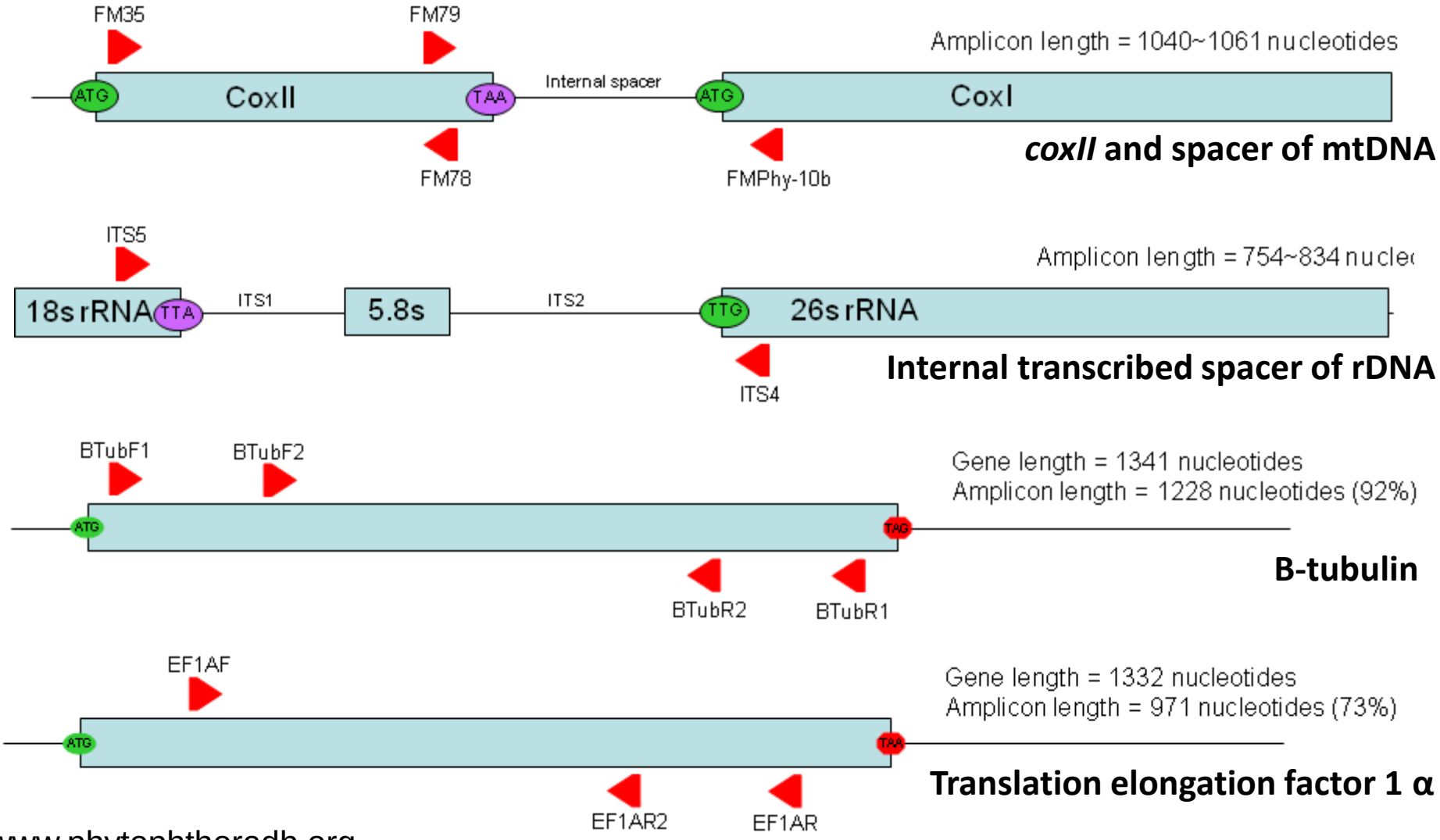
Molecular Identification

DNA sequencing

- Determine the exact sequence of As, Ts, Gs and Cs in a particular region of DNA

- Often use the ITS region – good for distinguishing species – especially for fungi and Oomycota

Loci used for DNA Identification



www.phytophthoradb.org

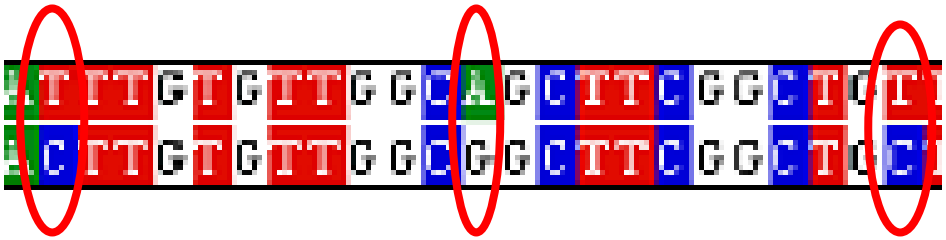
Molecular Identification

DNA sequencing

ITS

P. lateralis

P. ramorum



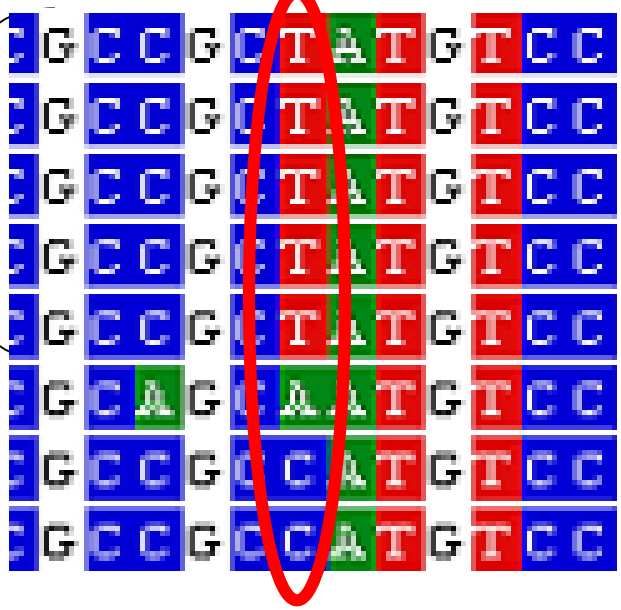
Beta-Tubulin

P. ramorum

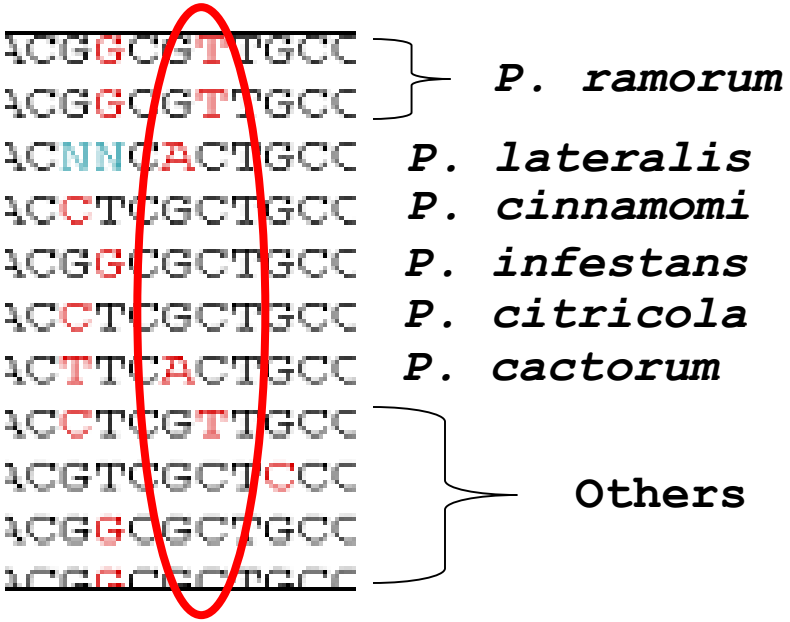
P. lateralis

P. cactorum

P. cinnamoni



Elicitin



Molecular Identification

DNA sequencing

- Determine the exact sequence of As, Ts, Gs and Cs in a particular region of DNA
- Often use the ITS region – good for distinguishing species – especially for fungi and Oomycota
- **Use the sequence to:**
  - **Identify an unknown organism**
    - **Using GenBank or the *Phytophthora* Database**

Molecular Identification

DNA sequencing

>gi|183013890|gb|EU427473.1|
ATGGAAGGTATTATTAACTTTCACCATGATTTAATGTTTTTTTTTAATTATGATTACTGTTTTGTTTTTGGA
TGTTATTAGAGTTATTACTCTTTTTGATGAAAAAAAAAATAAAATTCCTTCAACGGTAGTACATGGCGC
T
ACTATTGAAATTATTGGACATCTATTCCAGCTTTAATTTTATTAGTTGTTGCAGTACCATCTTTTGCTTT
A
TTATATTCAATGGATGAGGTAATTGATCCAATTATTACATTAAAAGTAATTGGTAGTCAATGGTATTGGA
G
TTATGAATATTCTGATAATTTAGAATTTTCTGATGAACCTTTAATTTTTGATAGTTACATGATACAAGAAG
ATGATTTAGCAATAGGTCAATTTAGAGTTTTAGAAGTAGATAATCGTGTAGTTGTACCAACAAATAGTC
AT
ATTAGAGTATTAATTACCGCATCAGATGTTTTACATTCATGGGCTATTCCTTCATTAGGTATTAAATTAG
A
TGCATGTCCTGGACGTTTAAATCAAACATCAATGTTTATTAAAAGAGAAGGTGTTTTTTATGGACAAT
GT
AGTGAAATTTGTGGAGTAAATCATGGATTTATGCCTATTGTTGTAGAAGCTGTTTCATTAGAAGATTAT
TT
AACTTGGTTAAAAAATAAAATCAATTTTGATTTTAATGTATAATGATTTTAATGTATAATTAAATTAATTT
TATGGTATTTAAAATCATGGGTGTAATTTGTTTAATATTATTATTATTTACAGATATTAAACAAATTATATA
TAAAATCAAACAATTTTTTAATAAATAAAAAAATATTATCAATGATAATATAAATTAAAAAAACCAACGC
TTTTTTTAATTAAAAAAATATATAATTTTGCATTTAAATTAAATTTTAAAAATATTCAAAA

www.Phytophthoradb.org

Phytophthora DATABASE
WWW.PHYTOPHTHORADB.ORG

Search :: Search by sequence

Marker

Internal Transcribed Spacer 1 & 2

- 60S Ribosomal Protein L10
- Beta Tubulin
- Enolase
- Heat Shock Protein 90
- Internal Transcribed Spacer 1 & 2
- Large Subunit rRNA
- Mitochondrial Cox
- TigA gene fusion
- Translation Elongation Factor 1 alpha

Sequence (FASTA)

» or Browse...

Limit Expect Value 1e-30

Number of matching sequences 20

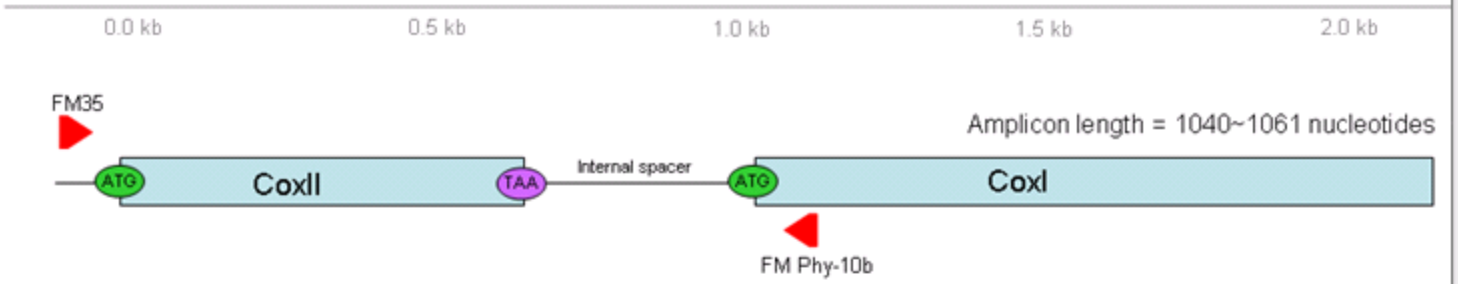
Matrix BLOSUM62

[Submit] [Back]

Phytophthora DATABASE
WWW.PHYTOPHTHORADB.ORG

Mitochondrial Cox

Cytochrome oxydase II gene(COXII) and Internal spacer



PCR Amplification Conditions & Primers

Forward: FM35	CAG AAC CTT GGC AAT TAG G	Position:-44~-22 from CoxII
Reverse: FM Phy-10b	GCA AAA GCA CTA AAA ATT AAA TAT AA	Position:+72~+97 of CoxI

Sequencing primers: same as FM35 and FM Phy-10b

(Primers from Martins et al. (2004) Phytopathology 94:621-631)

PCR conditions:
Epicentre FailSafe PCR 2X premix **A** buffer
Primers 0.01 µM
Taq 2 Unit
Template DNA 5 ng

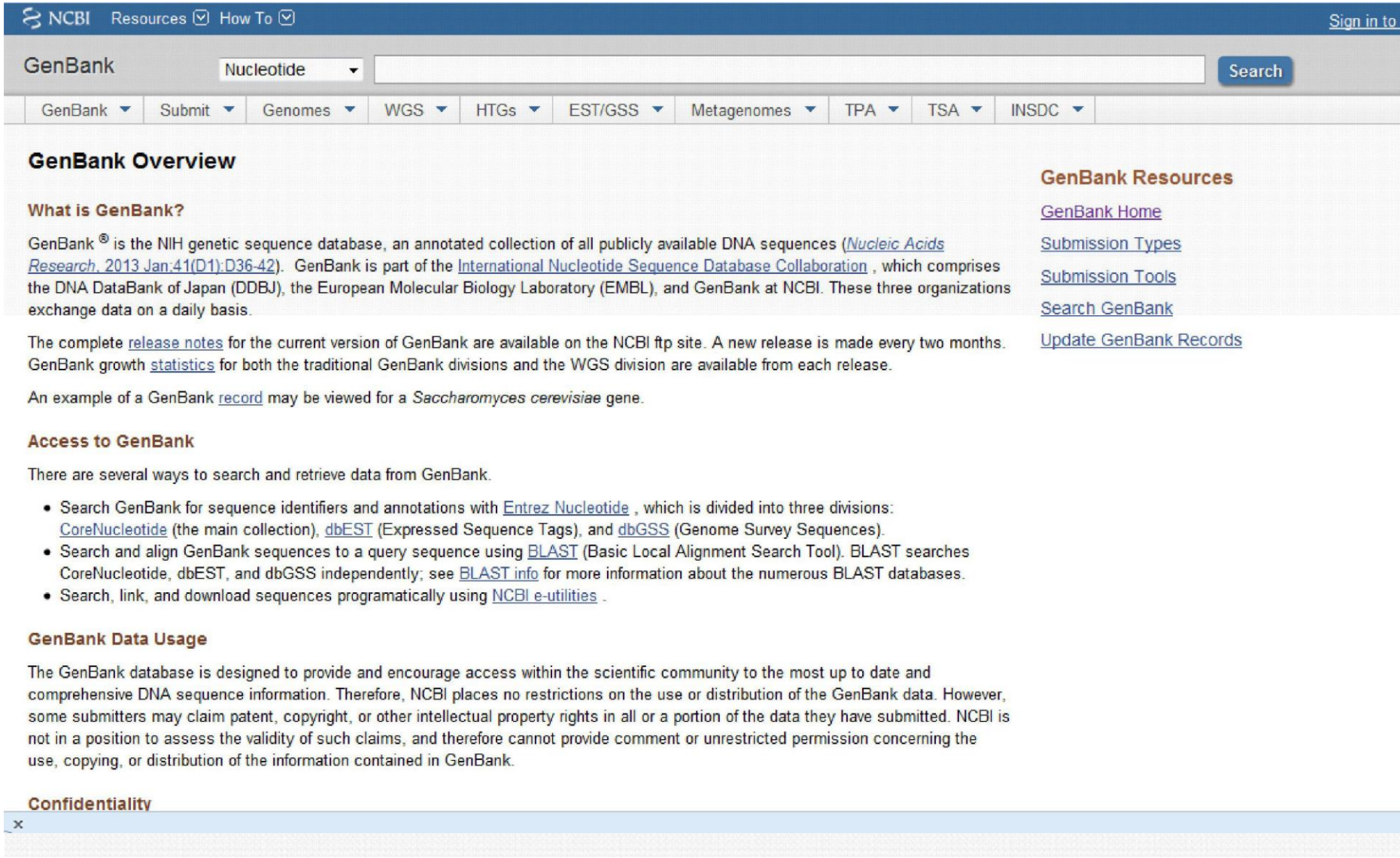
Program:
3 min 94°
1 min 94° |
1 min 47° | 35 cycles
1 min 30sec 72° |
5 min 72°

Diseases

Because there are over 100 + host species for P. ramorum at current, there are many different types of disease symptoms associated with the pathogen.
Symptoms on true oaks (Quercus spp.) and tanoak (Lithocarpus densiflorus) include blackened trunk / bole cankers, often with burgandy-colored ooze protruding from the bark (bleeding cankers). Cankers typically occur in the lower 10 ft of the trunk and are restricted to above the soil line. On many oak species, tree mortality often ensues, but usually not as sudden as the disease name suggests. Leaves may cling to branches up to one year after tree death.

Tanoak canker.

Genbank


NCBI Resources How To
Sign in to
GenBank
Nucleotide
Search
GenBank Submit Genomes WGS HTGs EST/GSS Metagenomes TPA TSA INSDC
GenBank Overview
What is GenBank?
GenBank ® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (Nucleic Acids Research. 2013 Jan;41(D1):D36-42). GenBank is part of the International Nucleotide Sequence Database Collaboration , which comprises the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI. These three organizations exchange data on a daily basis.
The complete release notes for the current version of GenBank are available on the NCBI ftp site. A new release is made every two months. GenBank growth statistics for both the traditional GenBank divisions and the WGS division are available from each release.
An example of a GenBank record may be viewed for a Saccharomyces cerevisiae gene.
Access to GenBank
There are several ways to search and retrieve data from GenBank.
Search GenBank for sequence identifiers and annotations with Entrez Nucleotide , which is divided into three divisions: CoreNucleotide (the main collection), dbEST (Expressed Sequence Tags), and dbGSS (Genome Survey Sequences).
Search and align GenBank sequences to a query sequence using BLAST (Basic Local Alignment Search Tool). BLAST searches CoreNucleotide, dbEST, and dbGSS independently; see BLAST info for more information about the numerous BLAST databases.
Search, link, and download sequences programatically using NCBI e-utilities .
GenBank Data Usage
The GenBank database is designed to provide and encourage access within the scientific community to the most up to date and comprehensive DNA sequence information. Therefore, NCBI places no restrictions on the use or distribution of the GenBank data. However, some submitters may claim patent, copyright, or other intellectual property rights in all or a portion of the data they have submitted. NCBI is not in a position to assess the validity of such claims, and therefore cannot provide comment or unrestricted permission concerning the use, copying, or distribution of the information contained in GenBank.
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Molecular Identification

DNA sequencing

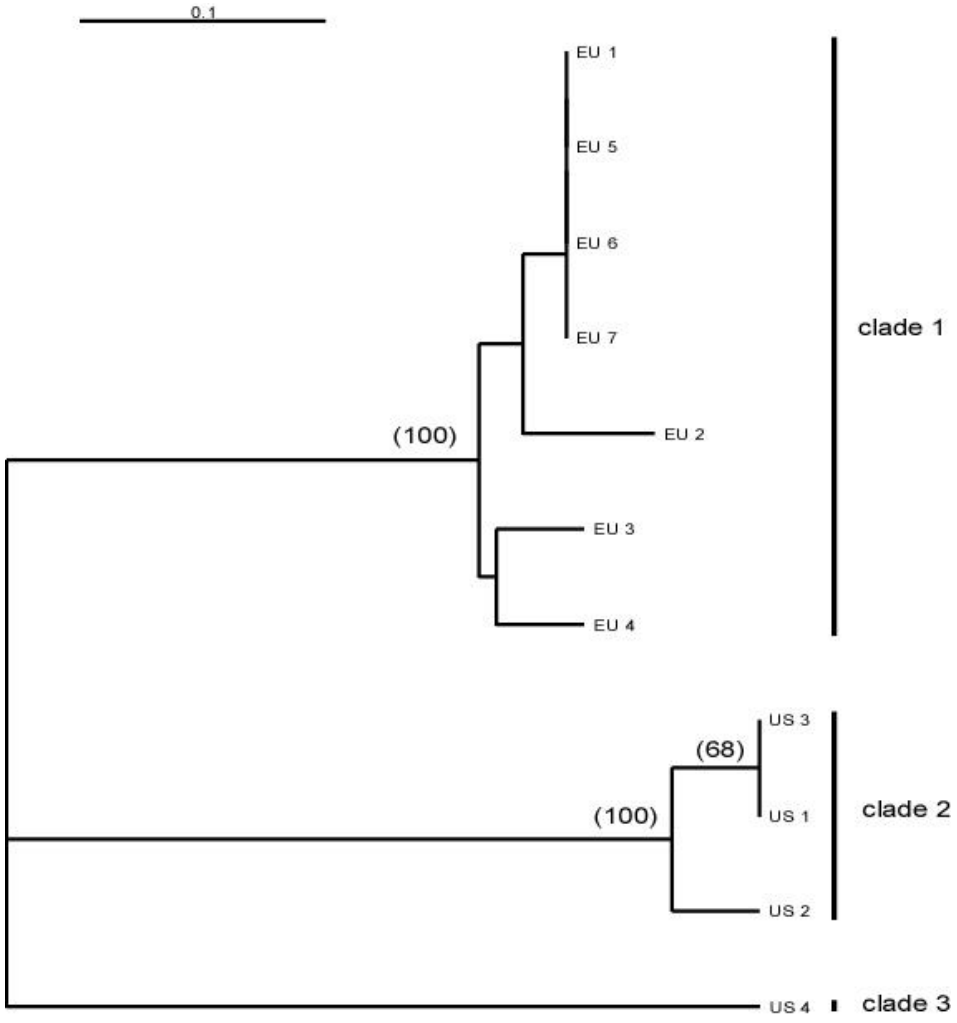
- Determine the exact sequence of As, Ts, Gs and Cs in a particular region of DNA

- Often use the ITS region – good for distinguishing species – especially for fungi and Oomycota

- **Use the sequence to:**
  - Identify an unknown organism
    - Using GenBank or the *Phytophthora* Database
  - **Compare with other organisms to infer relatedness**
    - **Alignment and Phylogenetic tree building**

Molecular Identification

Tree building



Molecular Diagnostics

- Detect (*in planta* / environmental samples)
- Quantify (*in planta* / environmental samples)
- Identify (*in vitro* / isolates)
- **Characterize population**
  - **determine strain or lineage**

Molecular Diagnostics

To answer these questions…

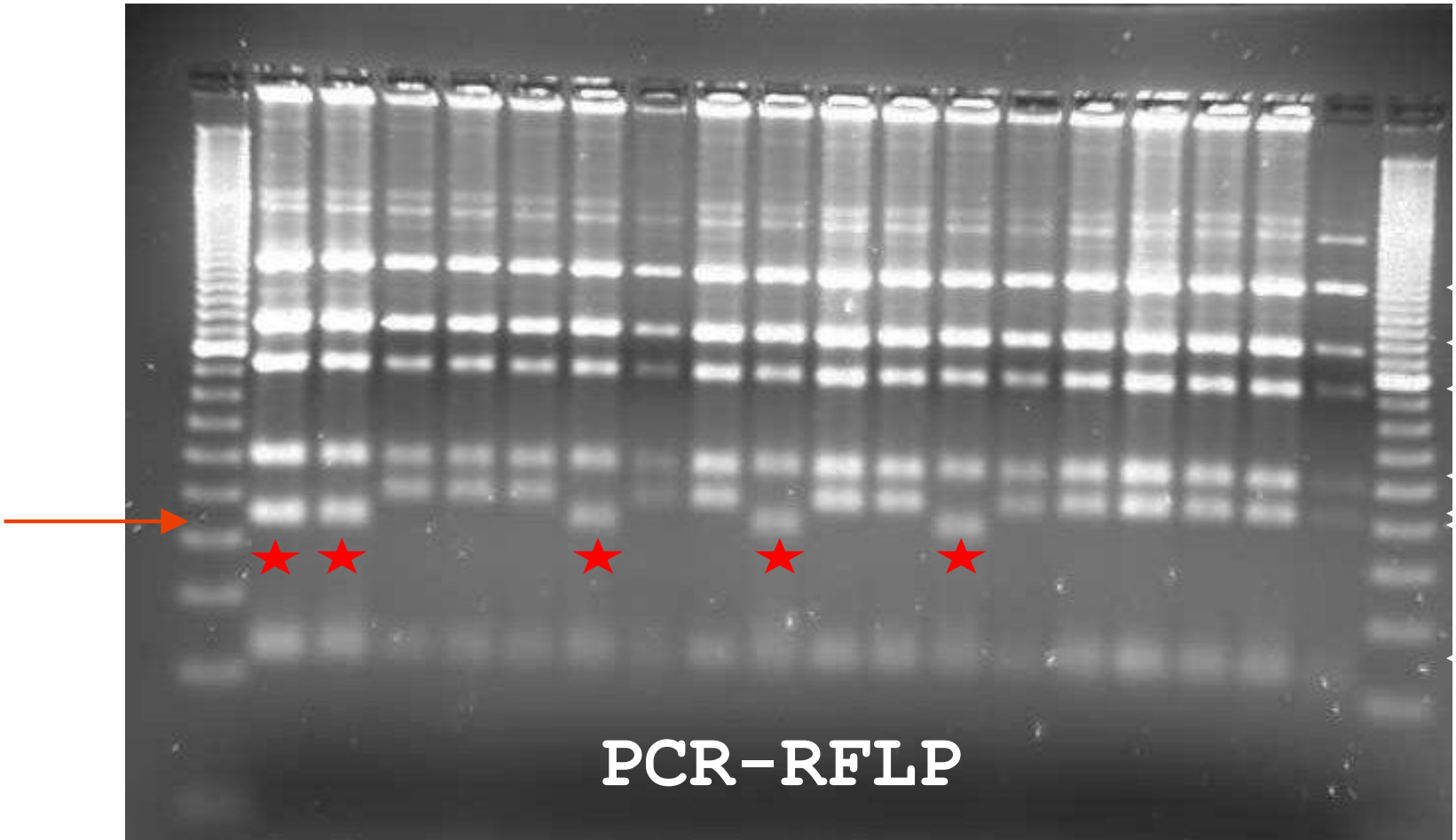
Gene sequence analysis Cox-I

***P.ramorum* Baysample**	AGGTCAAAT**T**CATTTTTGGTT
P.ramorum OakUS479	AGGTCAAAT**T**CATTTTTGGTT
P.ramorum RhodEU233	AGGTCAAAT**C**CATTTTTGGTT
P.ramorum RhodEU98	AGGTCAAAT**C**CATTTTTGGTT
P.ramorum ViburEU474	AGGTCAAAT**C**CATTTTTGGTT

Restriction enzyme: *Apo I*

Gene sequence analysis Cox-I

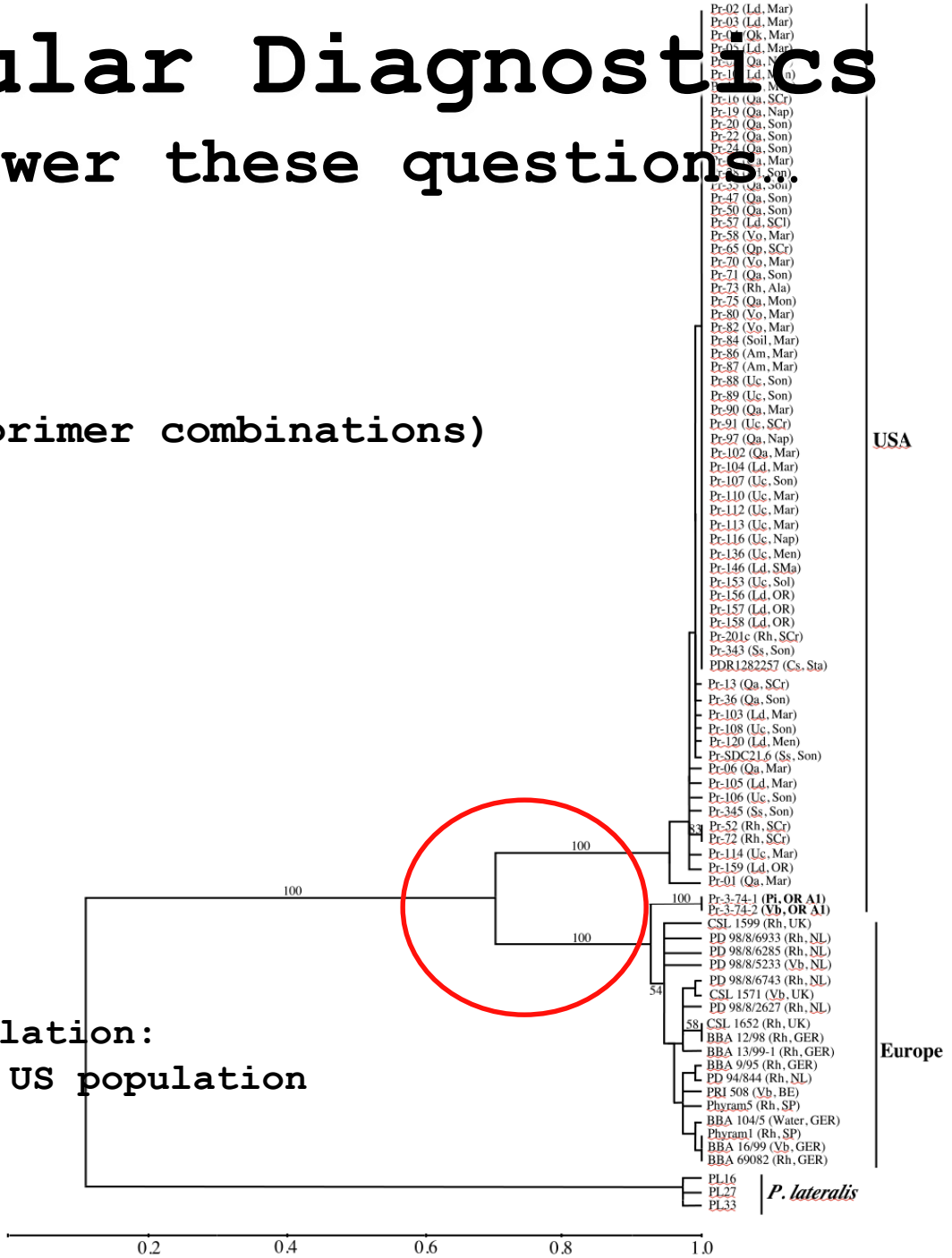
P. ramorum



Molecular Diagnostics

To answer these questions.

AFLP results (4 primer combinations)

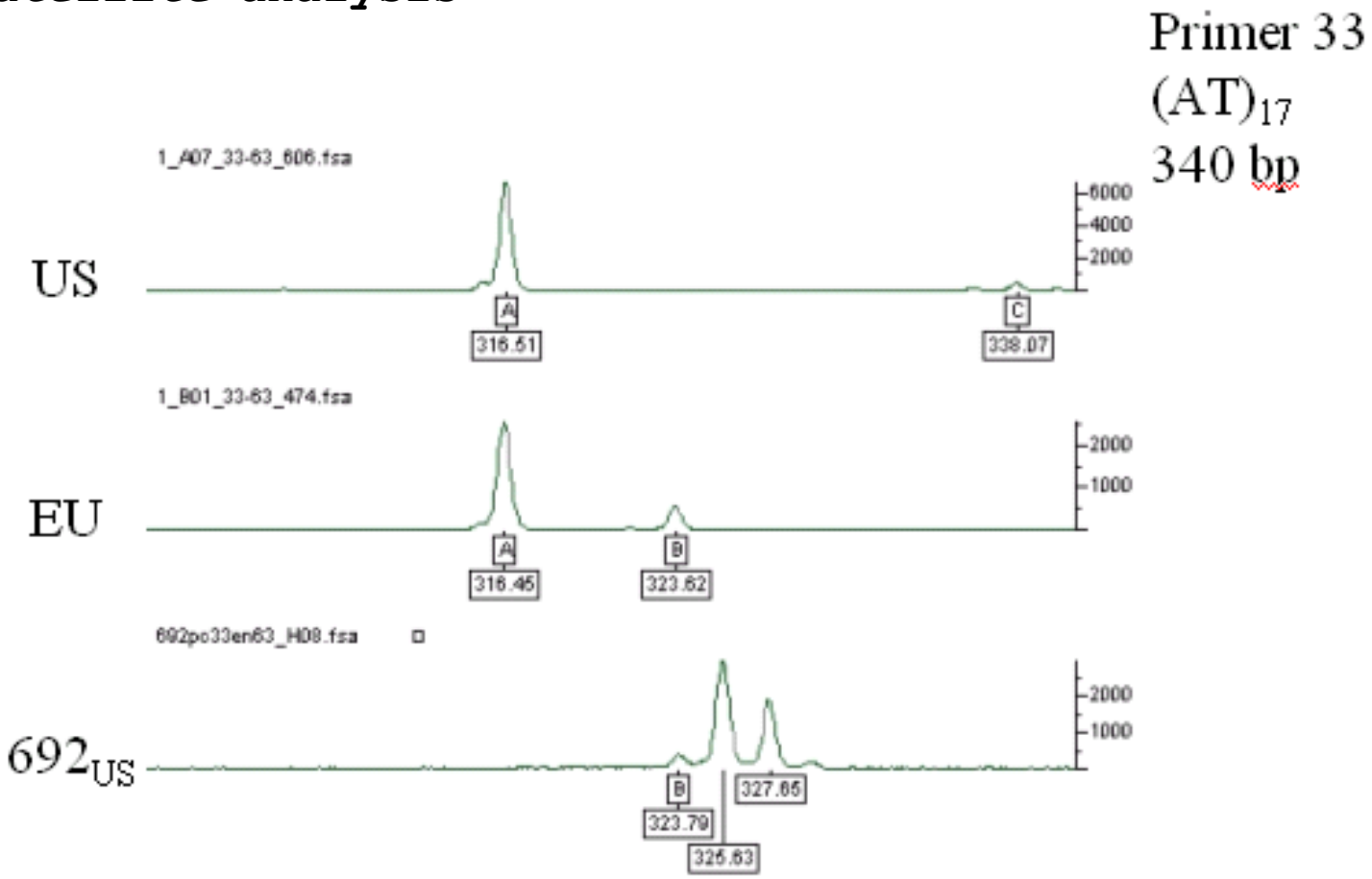


EU population:
Distinct from US population

Molecular Diagnostics

To answer these questions…

Microsatellite analysis



Molecular Diagnostics

To answer these questions…

Is Phytophthora species X present on the leaves?

Use species specific DNA primers or probe to detect specific DNA in the plant sample

What species of Phytophthora is infecting the leaves?

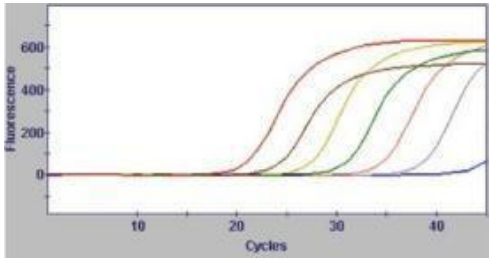
- Sequence a variable gene and compare it with other known isolates
- Build a tree and see which of the other sequences the unknown groups with – i.e. which is it most similar to

Molecular Diagnostics

To answer these questions…

Is P. ramorum present on the leaf?

- **Realtime PCR – PRESENCE/ABSENCE**

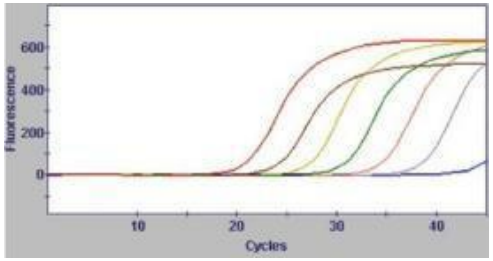


Molecular Diagnostics

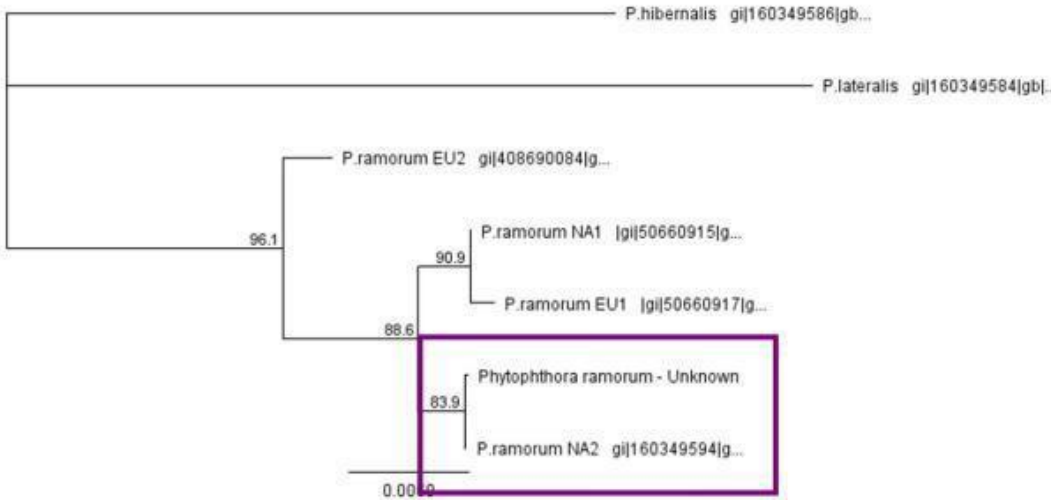
To answer these questions…

Is P. ramorum present on the leaf?

- **Realtime PCR – PRESENCE/ABSENCE**



What lineage does the unknown sample group with?



Molecular Diagnostics
summary

- Use different techniques depending on the questions asked
- Time consuming… depends on how you look at it
- Can be complicated by the nature of the sample
- Multiple techniques should be integrated when necessary

Questions?

Fighting Phytophthora

On-line protocols book

Laboratory Protocols for *Phytophthora* Species
Protocol 01-12.1 (November 2015)
http://dx.doi.org/10.1094/APSprotocols/Phytophthora/01-12.1
Contributed by Sabine Werres and Corina Junker, Julius Kühn-Institut (JKI), Braunschweig, Germany