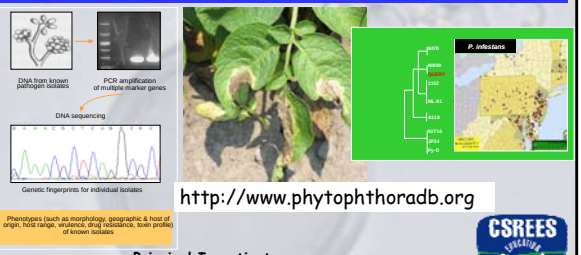
NC STATE Plant Pathology

Phytophthora identification with "The Phytophthora database"

Dr. Kelly Ivors
Dept. Plant Pathology
North Carolina State University
Mountain Hort. Crops Research & Extension Center
Mills River (Fletcher), NC

Phytophthora Database

Cyberinfrastructure supporting identification and monitoring of *Phytophthora* species



http://www.phytophthoradb.org

Principal Investigators
- Penn State: Seogchan Kang, David Geiser & Izabela Makalowska
- UC-Riverside: Mike Coffey
- North Carolina State University: Kelly Ivors
- USDA-ARS: Frank Martin & Kerry O'Donnell

CSREES USDA
USDA-NRI
Plant Biosecurity

Our knowledge on the diversity and ecology of Phytophthora is limited.

The rapid expansion of global trade and human travel has greatly accelerated the introduction of exotic pathogens and variants of endogenous pathogens.

Objectives of the Project

- ✓ To establish a comprehensive phylogenetic framework for the genus *Phytophthora*
- ✓ **To build an internet database that crosslinks the genotype, phenotype, and distribution of individual *Phytophthora* species/isolates (generate data for database)**
- ✓ To develop and optimize molecular diagnostic tools for detecting and identifying *Phytophthora*

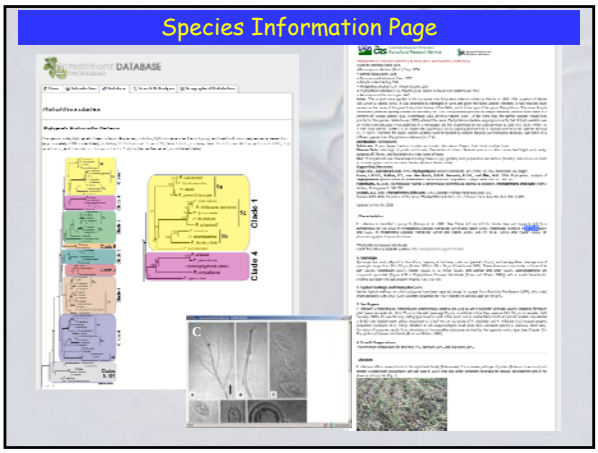
Park, J., Ivors, K., Kang, S. et al. 2008. *Phytophthora* Database: A forensic database supporting the identification and monitoring of *Phytophthora*. Plant Dis. 92:966-972.

Organization of the *Pytophthora* Database

www.Phytophthoradb.org

Park et al. Plant Disease (2008) 92: 966-972

Species Information Page



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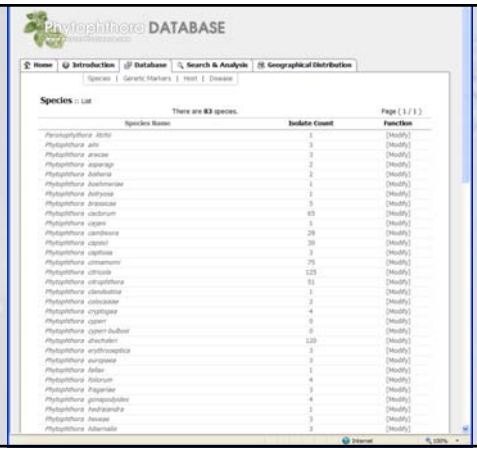

www.Phytophthoradb.org

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Life Cycle and Morphology

Semi-papillate sporangia of P. ramorum.

Pleroitc oospore with amphigynous antheridium of P. ramorum.

Known Diagnostics

Due to the high level of quarantine status of this pathogen, several detection techniques exist and many (not all) protocols have been validated by the USDA-APHIS for use by state agencies. For a summary of procedures approved by APHIS, please refer to the URL: http://www.aphis.usda.gov/ppq/ispm/pramorum/diagnosticsummary.html

Diagnostic protocols for P. ramorum can be divided into three different types of methods:
1). Isolation of P. ramorum onto selective media, followed by morphological identification of the pathogen microscopically. Most of the selective media used for P. ramorum isolation are amended with antibiotics, including Pimaricin, Ampicilin, Rifampicin, and PCNB.
2). DNA based detection of the pathogen. Several techniques exist including a nested-PCR protocol in the ITS region (Hayden et al., 05). Subgroeger and Tegtfen real-time PCR in the ITS region (Hayden et al., 06; Hughes et al., 06), and Taqman real-time PCR in the

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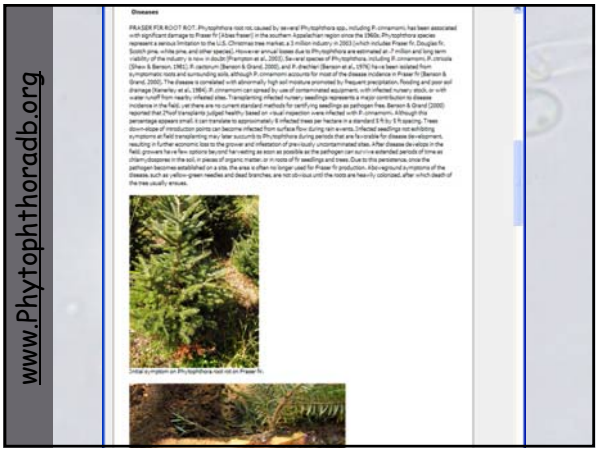

Diseases

FRASER FIR ROOT ROT: Phytophthora root rot, caused by several Phytophthora spp., including P. cinnamomi, has been associated with significant damage to Fraser fir (Abies fraseri) in the southern Appalachian region since the 1960s. Phytophthora species represent a serious limitation to the U.S. Christmas tree market, a 3 million industry in 2003 (which includes Fraser fir, Douglas fir, Scotch pine, white pine, and other species). However annual losses due to Phytophthora are estimated at .7 million and long term viability of the industry is now in doubt (Frampton et al., 2003). Several species of Phytophthora, including P. cinnamomi, P. cinnicola (Shaw & Benson, 1992), P. castorum (Benson & Grand, 2000), and P. drechsleri (Benson et al., 1976) have been isolated from symptomatic roots and surrounding soils, although P. cinnamomi accounts for most of the disease incidence in Fraser fir (Benson & Grand, 2000). The disease is correlated with abnormally high soil moisture promoted by frequent precipitation, flooding and poor soil drainage (Hanerley et al., 1984). P. cinnamomi can spread by use of contaminated equipment, with infected nursery stock, or with water runoff from nearby infested sites. Transplanting infected nursery seedlings represents a major contribution to disease incidence in the field, yet there are no current standard methods for certifying seedlings as pathogen free. Benson & Grand (2000) reported that 2%of transplants judged healthy, based on visual inspection were infected with P. cinnamomi. Although this percentage appears small, it can translate to approximately 6 infected trees per hectare in a standard 6 ft by 6 ft spacing. Trees down-slope of introduction points can become infected from surface flow during rain events. Infected seedlings not exhibiting symptoms at field transplanting may later succumb to Phytophthora during periods that are favorable for disease development, resulting in further economic loss to the grower and infestation of previously uncontaminated sites. After disease de-velops in the field, growers have few options beyond fan-wasting as soon as possible as the pathogen can survive extended periods of time as chlamydospores in the soil, in pieces of organic matter, or in roots of fir seedlings and trees. Due to this persistence, once the pathogen becomes established on a site, the area is often no longer used for Fraser fir production. Aboveground symptoms of the disease, such as yellow-green needles and dead branches, are not obvious until the roots are heavily colonized, after which death of the tree usually ensues.

Initial symptom on Phytophthora root rot on Fraser fir.

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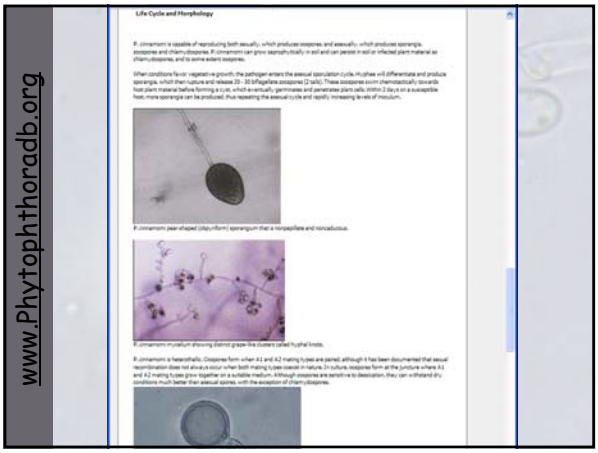

Life Cycle and Morphology

P. cinnamomi is capable of reproducing both sexually, which produces oospores, and asexually, which produces sporangia, zoospores and chlamydospores. P. cinnamomi can grow saprophytically in soil and can persist in soil or infected plant material as chlamydospores, and to some extent oospores.

When conditions favor vegetative growth, the pathogen enters the asexual sporulation cycle. Hyphae will differentiate and produce sporangia, which then rupture and release 20 - 30 biflagellate zoospores (2 tails). These zoospores swim chemotactically towards host plant material before forming a cyst, which eventually, germinates and penetrates plant cells. Within 2 days on a susceptible host, more sporangia can be produced, thus repeating the asexual cycle and rapidly increasing levels of inoculum.

P. cinnamomi pear-shaped (obpyriform) sporangium that is nonpapillate and noncaducous.

P. cinnamomi mycelium showing distinct grape-like clusters called hyphal knots.

P. cinnamomi is heterothallic. Oospores form when A1 and A2 mating types are paired, although it has been documented that sexual recombination does not always occur when both mating types coexist in nature. In culture, oospores form at the juncture where A1 and A2 mating types grow together on a suitable medium. Although oospores are sensitive to desiccation, they can withstand dry conditions much better than asexual spores, with the exception of chlamydospores.


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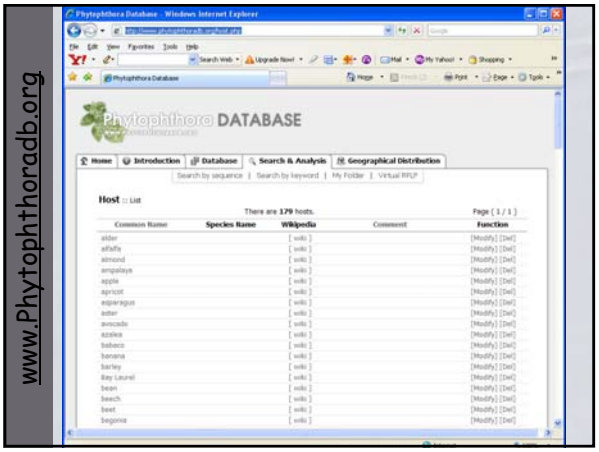
Known Diagnostics
Control Strategies
Acknowledgements
Notes
References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Phytophthora Database - Windows Internet Explorer
Phytophthora DATABASE
Home
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Host :: List
There are 179 hosts.
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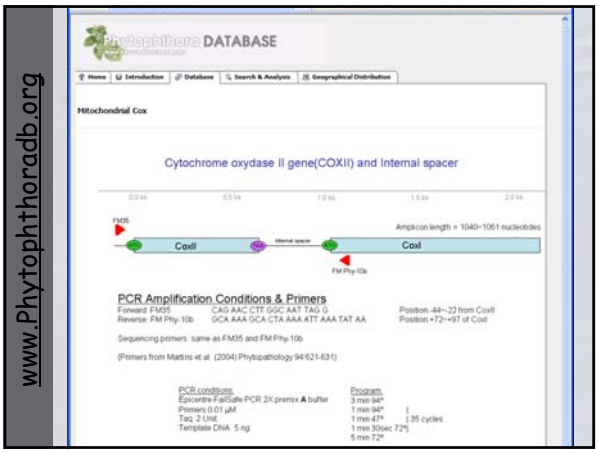
Common Name
Species Name
Wikipedia
Comment
Function


Arbutus
From Wikipedia, the free encyclopedia
Arbutus is a genus of flowering plants in the family Ericaceae, native to warm temperate regions of the Mediterranean, western Europe, and North America.
Scientific classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Ericales
Family: Ericaceae
Genus: Arbutus
Species
See text.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Phytophthora DATABASE
Home
Introduction
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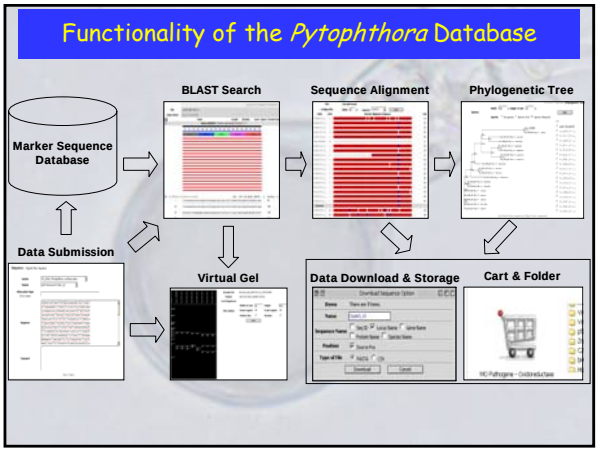
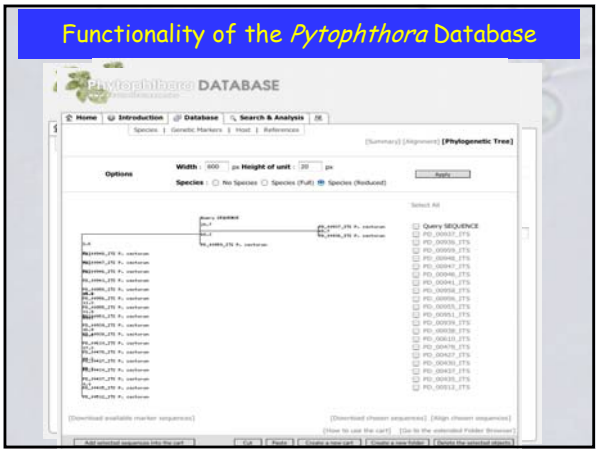
Geographical Distribution
Mitochondrial Cox
Cytochrome oxydase II gene(COXII) and Internal spacer
FM35
CoxII
CoxI
FM Phy-10b
Amplicon length = 1040~1061 nucleotides
PCR Amplification Conditions & Primers
Forward: FM35 CAG AAC CTT GGC AAT TAG G
Reverse: FM Phy-10b GCA AAA GCA CTA AAA ATT AAA TAT AA
Position -44~-22 from CoxII
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°
1 min 30sec 72°
5 min 72°
35 cycles


Functionality of the Pytophthora Database
Marker Sequence Database
BLAST Search
Sequence Alignment
Phylogenetic Tree
Data Submission
Virtual Gel
Data Download & Storage
Cart & Folder


Functionality of the Pytophthora Database
Phytophthora DATABASE
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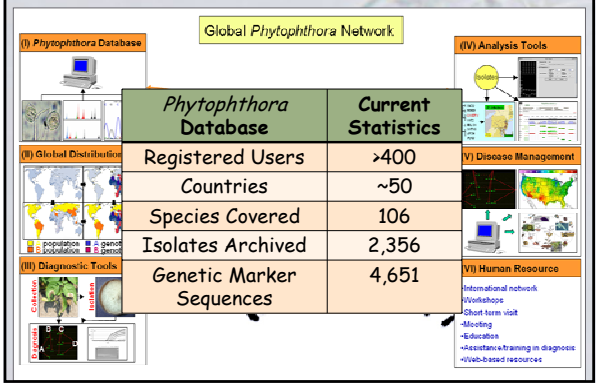
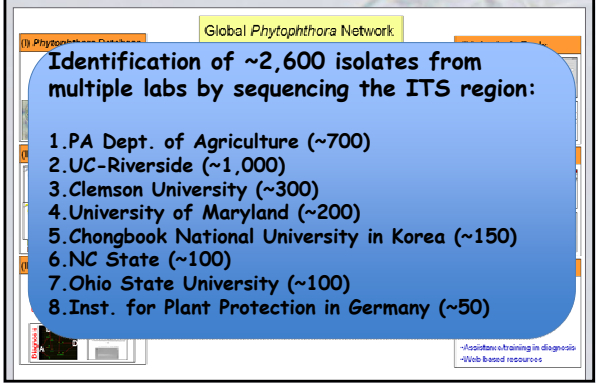
[Phylogenetic Tree]
Options
Width :
Height of unit :
Species :
No Species
Species (Full)
Species (Reduced)
Apply
Select All
Query SEQUENCE

Global *Phytophthora* Network



Phytophthora Database	Current Statistics
Registered Users	>400
Countries	~50
Species Covered	106
Isolates Archived	2,356
Genetic Marker Sequences	4,651

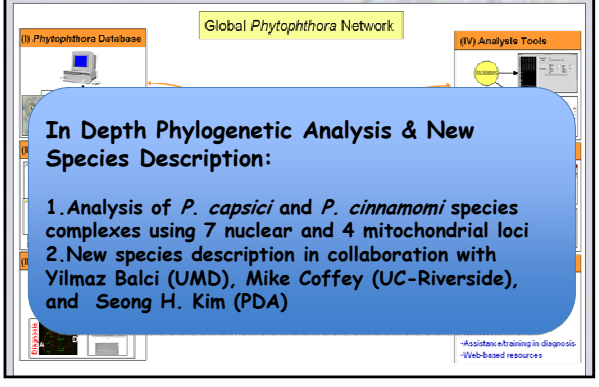
Global *Phytophthora* Network



Identification of ~2,600 isolates from multiple labs by sequencing the ITS region:

1. PA Dept. of Agriculture (~700)
2. UC-Riverside (~1,000)
3. Clemson University (~300)
4. University of Maryland (~200)
5. Chongbook National University in Korea (~150)
6. NC State (~100)
7. Ohio State University (~100)
8. Inst. for Plant Protection in Germany (~50)

Global *Phytophthora* Network



In Depth Phylogenetic Analysis & New Species Description:

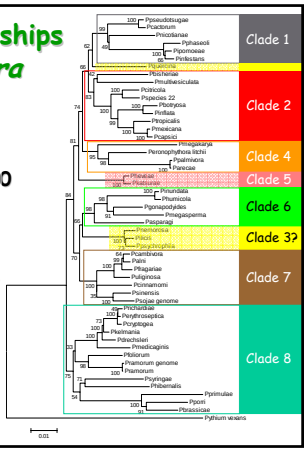
1. Analysis of *P. capsici* and *P. cinnamomi* species complexes using 7 nuclear and 4 mitochondrial loci
2. New species description in collaboration with Yilmaz Balci (UMD), Mike Coffey (UC-Riverside), and Seong H. Kim (PDA)

Phylogenetic Relationships Among *Phytophthora*

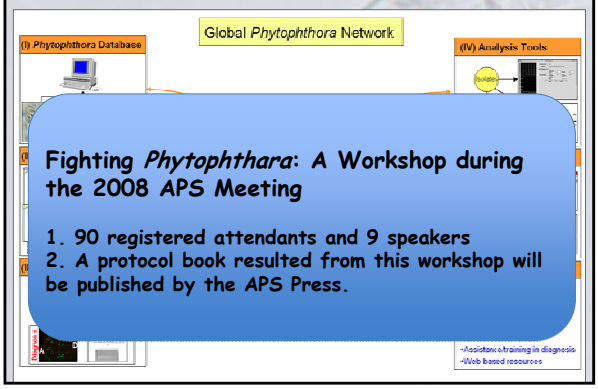
Five Protein-coding Loci:

- 60S Ribosomal Protein L10
- Beta-tubulin
- Enolase
- Heat Shock Protein 90
- Elongation Factor 1 alpha
- rRNA Large Subunit

Blair *et al.* 2008
www.phytophthoradb.org



Global *Phytophthora* Network



Fighting *Phytophthara*: A Workshop during the 2008 APS Meeting

1. 90 registered attendants and 9 speakers
2. A protocol book resulted from this workshop will be published by the APS Press.

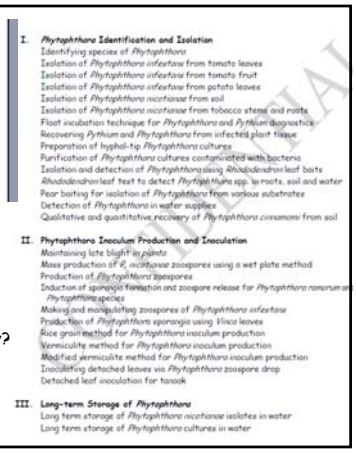
Fighting *Phytophthora*:

A Compilation of *Phytophthora*

Laboratory Protocols

Proposal submitted to APS and accepted

On-line information delivery?



Molecular Technology for Phytophthora identification and Development of Diagnostic Markers

Species-Specific Diagnostic Markers

Detailed Description of Diagnostic Markers

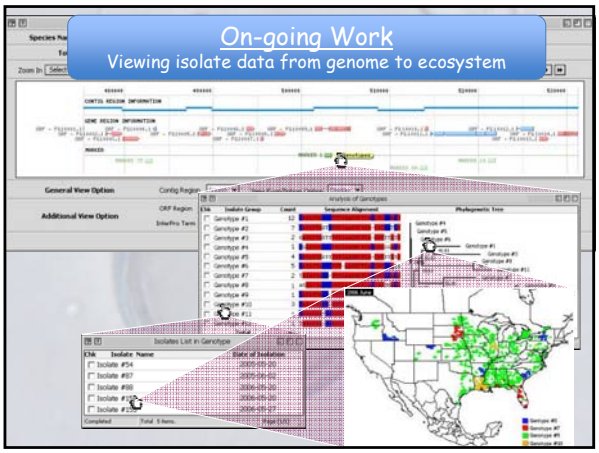
Sequence Alignment Files for Downloading

Reference Management System

Geographic Information Systems Tools

On-going Work

Viewing isolate data from genome to ecosystem



Future Direction

Cyber-infrastructure for *Phytophthora*

Phytophthora Database (PD)
http://www.phytophthoradb.org/

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Phytophthora Human Network