Phytophthora Root Rot on Fir
Research Findings from North Carolina and Beyond

John Frampton
Professor & Christmas Tree Geneticist
Department of Forestry & Natural Resources
North Carolina State University

CTFANY
2018 Winter Convention
Syracuse
January 19th
Presentation Outline

- Background Information
- Screening for resistance to *Phytophthora cinnamomomi*
- Other *Phytophthora* species
- Strategies
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North Carolina’s Christmas Tree Industry

- 2nd ranked state in U.S.
- 5-6 million trees harvested annually
- $100+ million wholesale value realized annually
- 2,500+ Christmas tree growers
- 98%+ of the harvested Christmas trees are Fraser fir produced in the western mountainous portion of the state
- 80%+ of the Fraser fir production is wholesale and shipped country-wide
North Carolina’s Christmas Tree Industry
Species List

- **Fraser fir** (*Abies fraseri*)
- **Virginia pine** (*Pinus virginiana*)
- **Eastern white pine** (*Pinus strobus*)
- **Eastern redcedar** (*Juniperus virginiana*)
- **Leyland cypress** (*x Cupressocyparis leylandii* 'Leighton Green')
- **Arizona cypress** (*Cupressus arizonica* var. *glabra* 'Carolina Sapphire' & 'Clemson Greenspire')
- **Atlantic white cedar** (*Chamaecyparis thyoides*)
- **Various spruces** (*Picea* spp.)
Phytophthora

- Name is Greek for “plant destroyer”
- Genus of water mold (Oomycetes)
- About 100 species (and counting)
- Can multiply exponentially
- Many species have mating strains designated as A1 and A2
- Several spore stages
- Zoospores infect conifer roots and have two unlike flagella to sense and swim toward host
Sporangium releasing zoospores. (Oregon State University)

Electron micrograph of *Phytophthora cinnamomi* zoospores encysting. (Giles Hardy)

Zoospore infection of host plant. (Jim Deacon)
Brief History of *Phytophthora cinnamomomi* in the Southeast U.S.

<table>
<thead>
<tr>
<th>Early 19th Century</th>
</tr>
</thead>
</table>
| ❖ *Pc* believed to be introduced on exotic flora imported through southern ports  
| ❖ Reports of American chestnut and chinkapin mortality from lower elevations due to a root disease  

| 1930 | 1<sup>st</sup> definitive report of *Pc* in the U.S. on three rhododendron species  
| 1932 | *Pc* identified as cause of “ink disease” on American chestnuts  
| 1940s | Littleleaf emerged as important disease on shortleaf pine  
| 1963 | 1<sup>st</sup> Report of *Pc* on Fraser fir in North Carolina  
| 1976-1977 | Fraser fir survey – 10% of sites infested 
| | NC Christmas tree industry < 1 million trees  
| 1997-1998 | Fraser fir survey – 9% of sites infested 
| | NC Christmas tree industry 6-7 million trees  

Impact on Fraser Fir

- Primarily caused by *P. cinnamommi*
- Results in $6-9 million in revenue losses annually
- Control with Subdue feasible in nursery beds
- Site selection & clean planting stock are our only preventative measures for the field
- No resistance found in Fraser fir although other fir species have some resistance
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Resistance Screening in Fraser Fir

Complete mortality in two greenhouse inoculations studies:

- 100 open-pollinated families from Fraser fir seed orchard from Roan Mountain (1999)
Resistance Screening in Fraser Fir

Complete mortality in two greenhouse inoculations studies:

• 100 open-pollinated families from Fraser fir seed orchard from Roan Mountain (1999)
• 99 open-pollinated families from all six of provenances of Fraser fir (2003)
Resistance Screening in Fraser Fir

- Little, if any resistance in Fraser fir
- Other fir species may offer resistance
Variation in Resistance to *P. cinnamomi* among *Abies* Species

Objectives:
- Rank the relative resistance of fir species
- Identify potential sources of fir resistance
The Genus *Abies*

- 39-55 Species – 2nd largest genus in Pinaceae
- Temperate and frigid regions
- Sea-level to over 5,000 m elevation
- Northern Hemisphere - 14°N to 67°N latitude

Worldwide distribution of the genus *Abies* (Farjon 1990)
Methods

- Grew seedlings in greenhouse for two or three years
- Inoculated with rice grains colonized with *P. cinnamomi*
- Placed into an outdoor lath house and recorded mortality every two weeks for a total of 16 weeks
- 32 *Abies* species, 50 seed sources
- 6,629 seedlings total
Time from Inoculation (wks)

Mortality (proportion)

Mean

A. bornmuelleriana

A. firma

A. fraseri

A. pindrow
# Variation among *Abies* Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. firma</em> (momi)</td>
<td>11.3</td>
</tr>
<tr>
<td><em>A. pindrow</em> (pindrow, Himalayan)</td>
<td>30.0</td>
</tr>
<tr>
<td><em>A. bornmuelleriana</em> (Turkish)</td>
<td>61.3</td>
</tr>
<tr>
<td><em>A. cilicica</em> (Cilcican, Toros)</td>
<td>63.2</td>
</tr>
<tr>
<td><em>A. siberica</em> (Siberian)</td>
<td>68.8</td>
</tr>
<tr>
<td><em>A. borisii-regis</em> (King Boris)</td>
<td>75.0</td>
</tr>
<tr>
<td><em>A. nordmanniana</em> (Nordmann)</td>
<td>77.0</td>
</tr>
<tr>
<td><em>A. cephalonica</em> (Greek)</td>
<td>82.5</td>
</tr>
<tr>
<td><em>A. chensiensis</em> (Chinese)</td>
<td>82.5</td>
</tr>
<tr>
<td><em>A. equi-trojani</em> (Trojan)</td>
<td>84.2</td>
</tr>
<tr>
<td><em>A. concolor</em> (concolor, white)</td>
<td>84.4</td>
</tr>
</tbody>
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<th>Species</th>
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<tr>
<td><em>A. guatamalensis</em></td>
<td>90.2</td>
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<tr>
<td><em>A. fabri</em></td>
<td>90.6</td>
</tr>
<tr>
<td><em>A. alba</em></td>
<td>91.3</td>
</tr>
<tr>
<td><em>A. ernestii</em></td>
<td>91.9</td>
</tr>
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<tr>
<td><em>A. balsamea</em></td>
<td>98.6</td>
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<tr>
<td><em>A. lasiocarpa</em></td>
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<td><em>A. amabilis</em></td>
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</tr>
<tr>
<td><em>A. magnifica</em></td>
<td>100.0</td>
</tr>
<tr>
<td><em>A. nephrolepis</em></td>
<td>100.0</td>
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</table>
Conclusions

- Most fir species are highly susceptible to *P. cinnamomomi*
- Some resistance exits in the Mediterranean and central Asia regions
- Momi and West Himalayan (pindrow) fir appear to be relatively resistant
Caveats

- Technique may overlook some types of resistance
  - Young trees
  - Severe disease conditions
    - Roots confined
    - High inoculum load
    - Continuously wet medium
- Some mortality may not be due to *Phytophthora*
- Limited sampling of provenances within species
Three Most Resistant Species

• 1\textsuperscript{st} Momi fir
  – Poor Christmas tree quality
  – Breaks bud about one month earlier than Fraser fir
• 2\textsuperscript{nd} Pindrow fir
  – No little known about this species
  – Very long needles
  – Does not produce many buds on branches
• 3\textsuperscript{rd} Turkish fir
  – Closely related to Nordmann fir
  – Used as a Christmas tree in Europe & North America
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2005 Cone Collection Trip

Trojan Fir
\((\text{Abies equi-trojani})\)
1  Kazdagi
2  Can

Turkish Fir
\((\text{Abies bornmulleriana})\)
3  Uludag
4  Akyazi
5  Bolu
6  Safranbolu

6 Provenances
20 Trees/Provenance
----- ---------------------
120 Trees Total
Turkish Fir Seeds

Trojan Fir
(*Abies equi-trojani*)
1 Kazdagi
2 Can

Turkish Fir
(*Abies bornmulleriana*)
3 Uludag
4 Akyazi
5 Bolu
6 Safranbolu

6 Provenances
20 Trees/Provenance
----- -----------------------
120 Trees Total

Black Sea

Image © 2005 MDA EarthSat

Pointer 39°54′18.16″ N 30°12′11.99″ E elev 3304 ft Streaming [ ] 100% Eye alt 389.80 mi
Geographic Pattern of Resistance

- Although *P. cinnamomii* is not believed to be native to Turkey, other *Phytophthora* species are present (Balci and Halmschlager 2003)
- Current or past contact with *Phytophthora* or other Oomycete species
- Adaptation to environmental factors such as rainfall, soil temperature, and/or soil characteristics (e.g., texture)
A model (generalized estimation equations) was fit to all data of the seedlings inoculated from both species to test the fixed effects (species and provenance) over time. We modeled the probability of mortality ($\pi$) while accounting for random replication and family effects and repeated measure:

$$\eta_{ijklmn} = \log \frac{\pi}{1 - \pi} = \mu + R_i + S_j + P(S)_{lk} + R/S_{ij} + R/P(S)_{ik} + FS P(S)_{ljk} + b_1 W_n + b_2 W/S + b_3 S(P)_{jn} + b_4 W/S + b_5 W/S/R(jn) + b_6 W/S/R(lj) + b_7 W/S/R(lkj) + b_8 W/S/R(FP)_{ljk} + b_9 W/S/R(FP)_{lkj} + b_{10} W/S/R(FP)_{lkj} + e_{ijklmn}$$

where,

- $\eta_{ijklmn}$ is the link function $[g(\mu)]$ of mortality of the $n$th time of the $m$th seedling, $j$th species, $k$th provenance, $l$th family, and in the $i$th replication
- $\log \left[ \frac{\pi}{1 - \pi} \right]$ is the logit value or log of odds of mortality
- $\pi$ is the probability of mortality
- $\mu$ is the conditional mean
- $R_i$ is the $i$th replication effect,
- $i_0$ is the intercept,
- $S_j$ is the $j$th species effect,
- $P(S)_{lk}$ is the $l$th family effect of $j$th species,
- $R/S_{ij}$ is the interaction effect of $i$th replication and $j$th species,
- $R/P(S)_{ik}$ is the interaction effect of $i$th replication and $k$th provenance of $j$th species,
- $FS P(S)_{ljk}$ is the interaction effect of $i$th replication, $j$th species, and $k$th provenance,
- $b_1 W_n$ is the effect of $n$th week,
- $b_2 W/S$ is the interaction effect of $n$th week and $j$th species,
- $b_3 S(P)_{jn}$ is the interaction effect of $n$th week, $j$th species, and $k$th provenance,
- $b_4 W/S$ is the interaction effect of $n$th week and $j$th species,
- $b_5 W/S/R(jn)$ is the interaction effect of $n$th week, $j$th species, and $k$th provenance,
- $b_6 W/S/R(lj)$ is the interaction effect of $n$th week, $j$th species, and $l$th family,
- $b_7 W/S/R(lkj)$ is the interaction effect of $n$th week, $j$th species, $k$th provenance, and $l$th family,
- $b_8 W/S/R(FP)_{ljk}$ is the interaction effect of $n$th week, $j$th species, $k$th provenance, and $l$th family,
- $b_9 W/S/R(FP)_{lkj}$ is the interaction effect of $n$th week, $j$th species, $k$th provenance, and $l$th family,
- $b_{10} W/S/R(FP)_{lkj}$ is the interaction effect of $n$th week, $j$th species, $k$th provenance, and $l$th family,
- $e_{ijklmn}$ is the residual error.
## Family Means for Mortality

<table>
<thead>
<tr>
<th>Provenance</th>
<th># Families</th>
<th>Mean</th>
<th>Range of Family Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trojan fir (56.4%)</td>
<td>16</td>
<td>54.4</td>
<td>15.6-81.9</td>
</tr>
<tr>
<td>Turkish fir (32.9%)</td>
<td>18</td>
<td>59.8</td>
<td>36.2-92.5</td>
</tr>
<tr>
<td>Çan-Seed stand</td>
<td>20</td>
<td>51.1</td>
<td>10.7-88.7</td>
</tr>
<tr>
<td>Uludağ-NP</td>
<td>20</td>
<td>31.6</td>
<td>7.7-67.4</td>
</tr>
<tr>
<td>Akyazı-Dokurcun</td>
<td>12</td>
<td>22.7</td>
<td>7.9-88.7</td>
</tr>
<tr>
<td>Bolu-Kökez</td>
<td>19</td>
<td>21.4</td>
<td>0.0-45.5</td>
</tr>
<tr>
<td>Karabük-Safranbolu</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Heritability Estimates
16 Weeks after Inoculation

<table>
<thead>
<tr>
<th>Species</th>
<th>$h^2_{\text{Individual Tree}}$</th>
<th>$h^2_{\text{Family Mean}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trojan Fir</td>
<td>0.62 (0.162)</td>
<td>0.97 (0.011)</td>
</tr>
<tr>
<td>Turkish Fir</td>
<td>0.50 (0.102)</td>
<td>0.96 (0.010)</td>
</tr>
</tbody>
</table>
Conclusions

- Useful levels of resistance exist in Turkish and Trojan fir
- Turkish fir has a higher frequency of resistance than Trojan fir
- The frequency of resistance increase from west to east in the Trojan-Turkish fir range
- Resistance is under strong genetic control
Genetic Basis of Phytophthora Resistance in Trojan fir

Research of PhD graduate student, Will Kohlway
Funded by USDA Specialty Crops Grant

2 Approaches

Genotyping by Sequenceing (GBS)
  Genomic markers to select for resistant planting stock
  Does not address biological cause for resistance

RNA-seq
  “Snap-shot” of cellular response against Phytophthora
  Study gene expression patterns
  Identifies genes important to root rot resistance
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Phytophthora Species Survey

Research of PhD graduate student, Martin Pettersson

Funded by:
- Gunnar and Lillian Nicholson Graduate Fellowship and Faculty Exchange Fund
- NCSU Christmas Tree Genetics Program
- NIFA USDA SCRI
Growers have contracted out-of-state nurseries to produce their planting stock. This may have led to introduction of new Phytophthora species into the region. Katie McKeever (PhD, Washington State University) - isolated *P. cryptogea* (taxon *kelmania*) and *P. pini*.
Survey Christmas Tree Growers
(n=89 grower, 123 farms, 13 counties)

54% of growers surveyed use out-of-state planting stock
All large growers (>100 acres) surveyed use out-of-state planting stock
78% of acreage of surveyed growers planted with out-of-state planting stock
Most transplants originate from the Pacific Northwest
103 sites

309 diseased trees
32 healthy controls

682 petri dishes
Results

80% isolation success per site
54% isolation success per tree

6 species identified

- P. cinnamomi
- P. cryptogea (P. sp. kelmania)
- P. pini (P. citricola complex)
- P. europaea
- P. citrophthora
- P. sansomeana
Results

- P. cinnamomi: 71%
- P. cryptogea: 23%
- P. citrophthora: 2%
- P. europaea: 1%
- P. pini: 1%
- P. sansomeana: 1%
Summary

In this survey we found more *Phytophthora* species in Fraser fir Christmas tree plantations in the Southern Appalachians compared to previous published surveys.

While *P. cinnamomoi* remains the predominant species (71%), *P. cryptogea* appears to have become an important pathogen (23%) contributing to losses to the Christmas tree industry in the Southern Appalachian Mountains.
Summary

*P. citrophthora, P. europaea, P. pini* and *P. sansomeana* have not been reported in previously published Fraser fir surveys conducted in the region.

*P. citrophthora* that has only been found once before on an *Abies* species – Martin proved pathogenecity on Fraser fir (Koch’s Postulates)
New York Christmas Trees

Brian Eshenaaur & Shawn Kenaley – Cornell 2012
- *P. cryptogea/drechsleri*
- *P. catorum*
- *P. citricola*

Katie McKeever & Gary Chastagner WSU 2016
- *P. cryptogea* (taxon *kelmania*)
- *P. pulirivora*
- *P. pini*
- *P. catorum* (CT)
Interactions between Fir and Phytophthora

Research of MS graduate student, Will Kohlway
Funded by NCDA & CS Specialty Crops Block Grant

Objective: to determine differences in aggressiveness of specific genetic isolates of Phytophthora on various fir species.
Genetic Interactions between Fir and Phytophthora

4 Fir Species
- Fraser fir
- Momii fir
- Trojan fir
- Turkish Fir

7 Phytophthora Isolates

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>Mating Type</th>
<th>Host Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. cinnamomi</em></td>
<td>2322</td>
<td>A1</td>
<td>Camilla</td>
</tr>
<tr>
<td></td>
<td>2325</td>
<td>A2</td>
<td>Shore Juniper</td>
</tr>
<tr>
<td></td>
<td>2327</td>
<td>A2</td>
<td>Cedar</td>
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<tr>
<td></td>
<td>23ss04</td>
<td>A2</td>
<td>Fraser fir</td>
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<tr>
<td></td>
<td>23ss11</td>
<td>A2</td>
<td>Fraser fir</td>
</tr>
<tr>
<td><em>P. cryptogea</em></td>
<td>C161</td>
<td>A2</td>
<td>Fir</td>
</tr>
<tr>
<td></td>
<td>C198</td>
<td>A2</td>
<td>Fir</td>
</tr>
</tbody>
</table>
Difference in Agressiveness of *Phytophthora* Species

*P. cinnamomi*  
*P. cryptogea*
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Short-term Strategies

- Graft Fraser fir onto momi fir (*A. firma*) rootstock for deployment on infested sites
- Plant out resistant provenances (Bolu) of Turkish fir
Strategies

Intermediate

- Establish Turkish/Trojan fir seed orchard using survivors from past inoculation trials
- Study genetic control of resistance and develop DNA markers to select resistant trees from field trials (four in NC)

Long-term

- Produce genetically transformed resistant Fraser fir
- Breed for resistance within Turkish and other resistant fir species
- Develop resistant interspecific hybrids and backcrosses with Fraser fir
Planting Season

- Careful site selection
  - No past disease problems
  - Well-drained, low clay content
  - Minimize compaction during site prep

- Don’t plant Fraser fir on sites known to have *Phytophthora*
  - Fraser fir grafted onto resistant fir
  - Resistant fir
  - Spruce
  - White pine
Planting Season

- Carefully inspect all planting stock
- Shoot symptoms
  - Off-color
  - Low vigor / little growth
  - Short needles
- Root symptoms
  - Brown or black lesions
  - Dead roots
- Do not plant suspicious stock
- Have suspicious stock tested for Phytophthora
- Report positive results to Cooperative Extension Service
Phytophthora ImmunoStrips

25 = $145, $5.80 / test
**Phytophthora** ImmunoStrips

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7 Interpret Results

Remove test strip from extract and interpret results (see illustration).

If only the control line (C) is visible, this indicates a negative result.

If the test line (T) is also present at any intensity of pink/purple, this indicates a positive** result.

If no lines are present, the test is invalid (see troubleshooting).
THANKS !!!