Rapid PCR Pitch Canker Diagnostic Test

Ryan Nadel, Scott Enebak and Lori Eckhardt

Forest Health Dynamics Laboratory

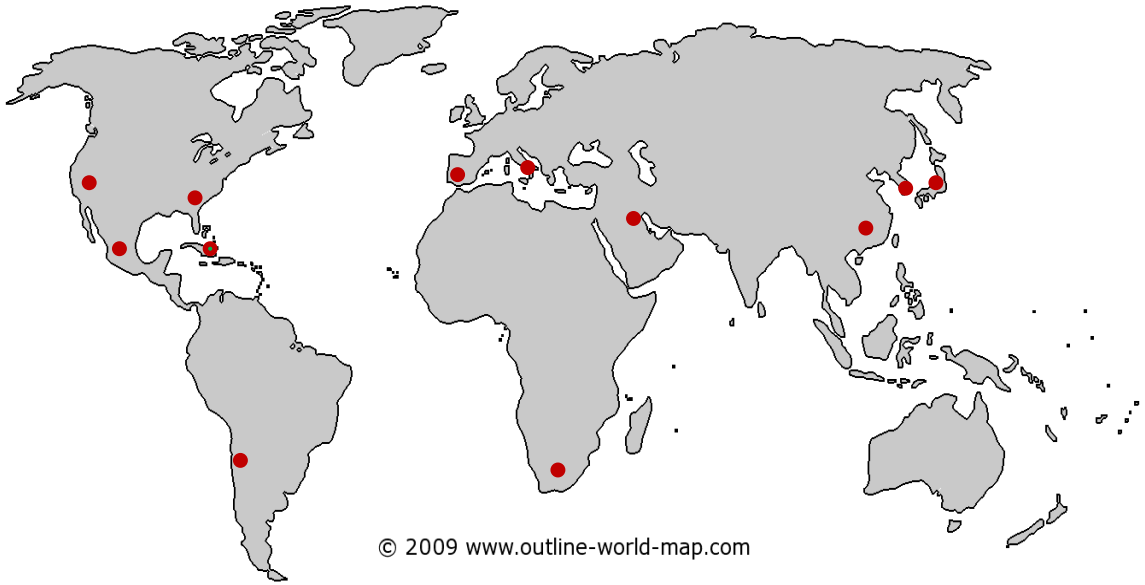
School of Forestry and Wildlife Sciences, Auburn University

Pitch Canker - Fusarium circinatum

- Pitch canker is caused by the fungal pathogen *Fusarium circinatum* (*Gibberella circinata*), a serious disease that affects numerous *Pinus* species grown Internationally.
- The term pitch canker refers to the resinous cankers that develop on roots, trunks, branches and cones of coniferous hosts.
- On seedlings, the pathogen mainly causes root and collar rot, damping-off and late season seedling blight.

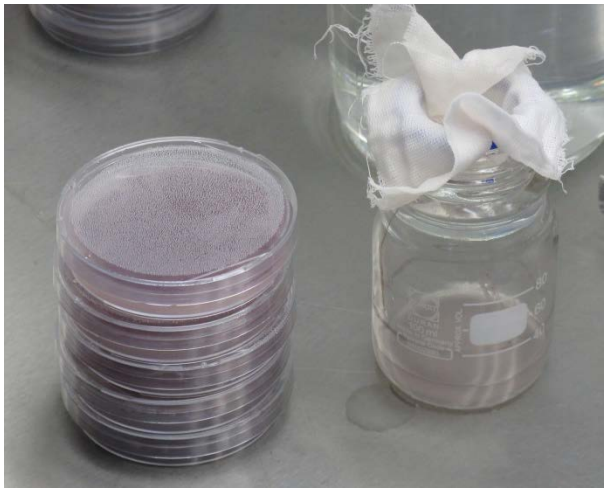
Fusarium circinatum

- The fungus probably originated in Hispaniola or Mexico and was introduced into the southern US.
- Since then, accidental introduction of *F. circinatum* has occurred in California, Asia, Europe, South Africa and South America.
- *Fusarium circinatum* internationally threatens plantation forestry that rely on susceptible pine species such as *Pinus radiata, P. patula* and *P. palustris.*



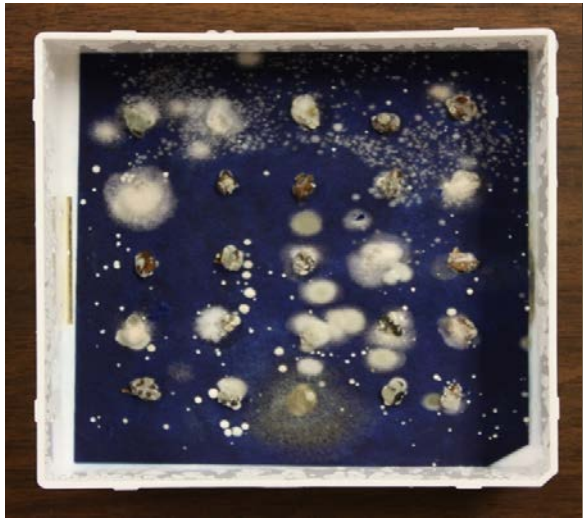
Seed and plant certification

- *Fusarium circinatum* is readily transported in and on *Pinus* seeds and plant cuttings.
- Seed certification, indicating the absence of the pathogen, is required for international seed importation/exportation.
- Currently the International Seed Testing Association (ISTA) accepts the seed screening blotter paper method that is used by the USDA Forest Service Resistance Screening Center.



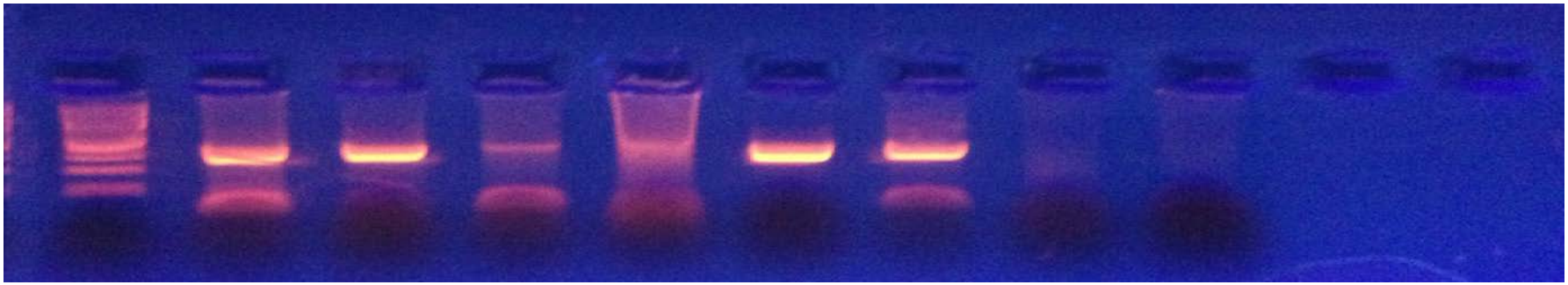
Seed screening method

- 400 seed per sample must be free of *F. circinatum*
- Relies on culturing the pathogen from seed on blotter paper infused with PCNB broth medium and identifying suspected colonies morphologically
- Does not allow for the reliable identification of suspected colonies to the species level
- The blotter paper method can also lead to false negative results, as numerous fungi may grow from the pine seed, covering *F. circinatum* colonies
- Time consuming - up to 6 wk



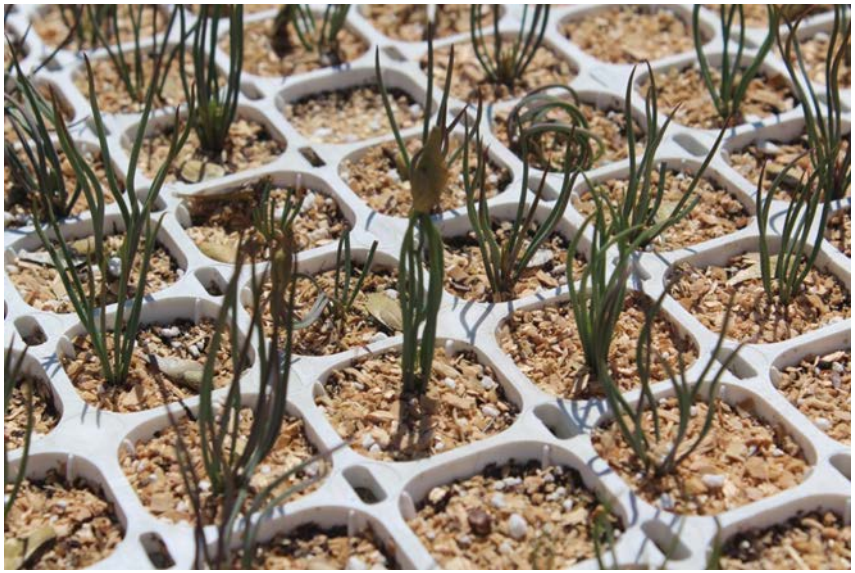
DNA extraction and PCR procedure

- A faster more accurate method resulting in bulk DNA extraction and a PCR procedure to screen seed for the presence of *F.* circinatum was needed.
- The objectives of this project
  - To identify species specific DNA primers for *F. circinatum.*
  - Develop a rapid screening protocol that will positively confirm either the presence or absence of the pathogen on pine planting material.



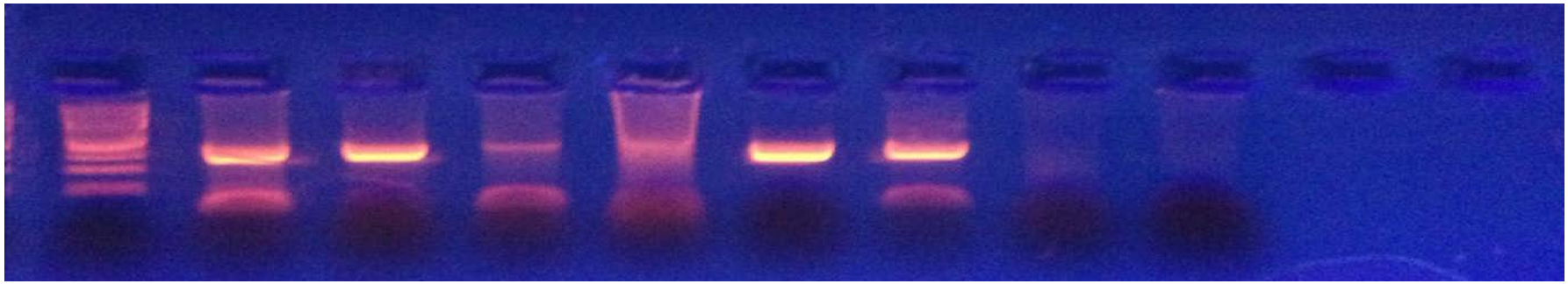
DNA extraction and PCR procedure

- We aim to compare *F. circinatum* contamination rates in a large number of seed lots across different pine species.
- The ability to quickly and positively identify the pathogen in seed lots and planting material will significantly reduce the spread and threat of this pathogen, both in the United States and Internationally.



Work to date

- Species specific primers were developed by our collaborators at the University of Florida.
- 360 bp piece of DNA that only works with *F. circinatum.*
- DNA extraction and PCR amplification protocols has been developed and refined.
- Seed sources have been obtained from across the southern US.
- Seed lots are currently being screened using both the blotter paper method and the DNA extraction and PCR amplification method.



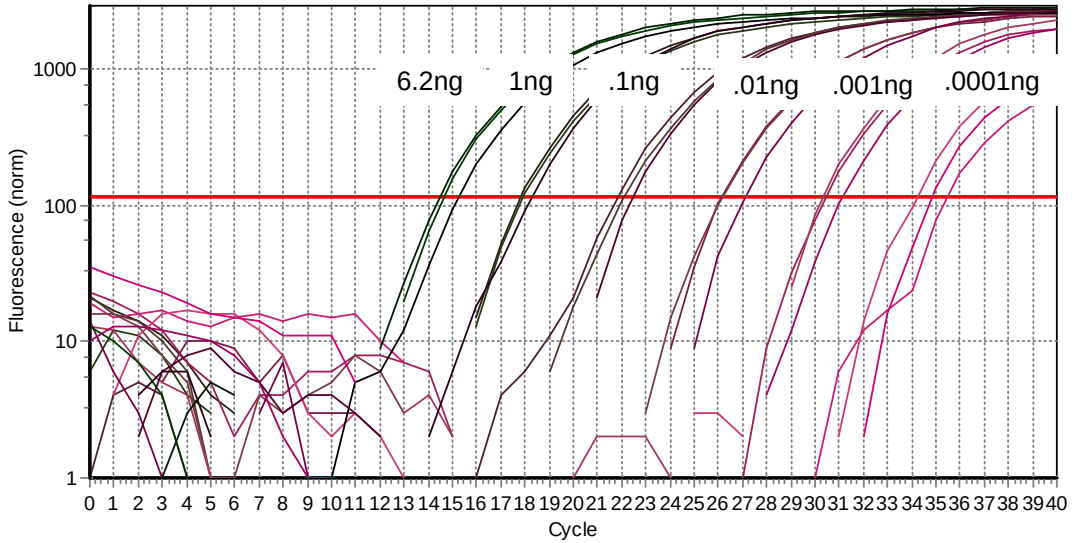
Species and collections to date:

Species	Families / Seedlots Collections / Sources	Members / Cooperators
Longleaf pine	49	5
Shortleaf pine	31	4
Loblolly pine	29	4
Slash pine	22	4
Pitch Pine	12	2
White Pine	11	4
Virginia pine	7	3
Sand pine	1	1

Impact of research

- This work will assist in reducing the spread and introduction of *F. circinatum* into new areas both within North America and Internationally.
- Improve the **speed** and **accuracy** in which *F. circinatum* can be identified from both seed and planting material.
- Rapidly detect *F. circinatum* in diseased plant materials.
- Compare cone/seed infestation/contamination for different sources.
- Use in epidemiology studies of *F. circinatum.*
- Screen seed lots for damping-off problems.
    - Yes or No infestation.
    - Quantification of infestation*?*

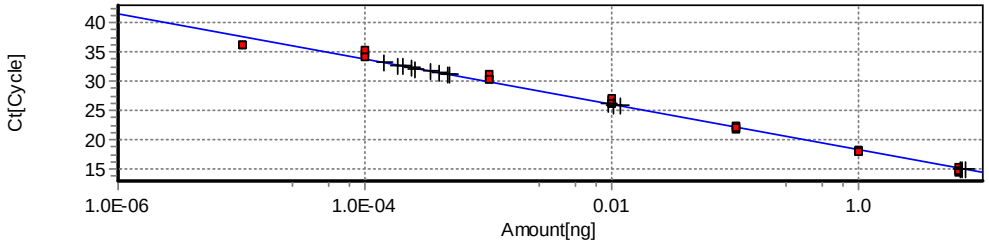
qPCR or How infested is the seed?



Cycle threshold (Ct)

Threshold: 117 (Noiseband)

Baseline settings: automatic, Drift correction OFF



Slope: -3.883

Y-Intercept: 18.32

Efficiency: 0.81

R^2: 0.988

Impact of research - already

- Member called and suspected seedling mortality due to *F. circinatum*
  - Sent Auburn samples of:
    - Unhealthy seedlings & Healthy seedlings
    - Seed from unhealthy seedlings and seed from “disease free” source
  - Arrived on Tuesday AM, and isolations were taken from seed and seedlings and plated onto selective media and remaining samples were process using new PCR test.
  - Wednesday noon
    - Unhealthy seedlings = positive for *F. circinatum*
    - Healthy seedlings = negative for *F. circinatum*
    - Seed from unhealthy seedlings = positive for *F. circinatum*
    - Seed from “disease free” seedlot = positive for *F. circinatum*
  - Five weeks later positively identified *F. circinatum* on seedlings and seed on the agar media

Acknowledgements

Rayonier

Southern Forest Nursery Management Cooperative

North Carolina Forest Service NC

Forest Service US Department of Agriculture

UF University of Florida

Georgia Forestry Commission

Florida Forest Service Agriculture & Consumer Services