Update: Rapid ID of Pitch Canker

Pitch canker

- Pitch canker is caused by the fungal pathogen *Fusarium circinatum* (*Gibberella circinata*), a serious disease that affects numerous *Pinus* species grown Internationally
- On seedlings, the pathogen mainly causes root and collar rot
- The term pitch canker refers to the large resinous cankers that develop on roots, trunks, branches and reproductive organs of mature pine tree hosts

Fusarium circinatum

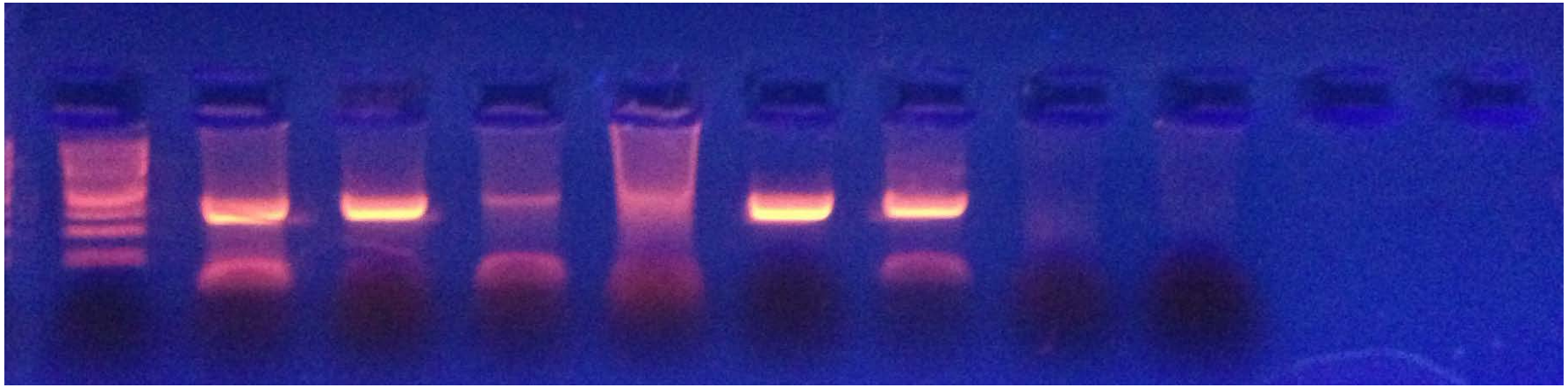
Images: Scott Enebak

Rapid identification

- Seed certification, indicating the absence of the pathogen is required for international seed importation
- Currently the International Seed Testing Association (ISTA) seed screening blotter paper method is used by the USDA Forest Service Resistance Screening Center to screen for the pathogen

DNA extraction and procedure

- A faster more accurate method was developed resulting in bulk DNA extraction and a PCR procedure to screen seed for the presence of *F. circinatum*
- The objectives of this study
  - To identify species specific 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

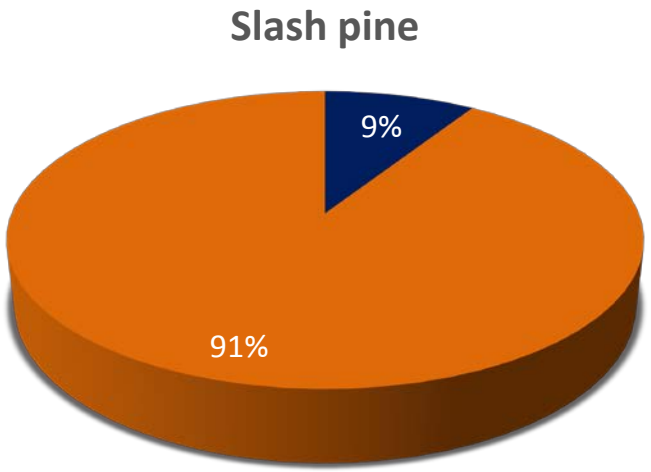
- We compared *F. circinatum* contamination rates in a large number of seed lots across different pine species
- The ability to quickly and positively identify the pathogen in seed lots and planting material will significantly reduce the spread and threat of this pathogen, both in the United States and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

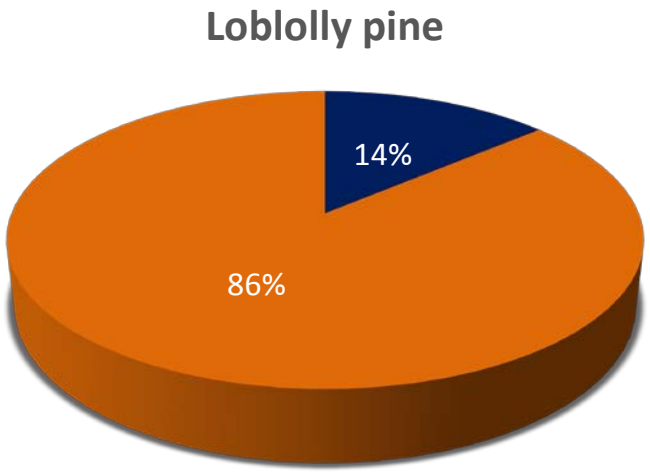


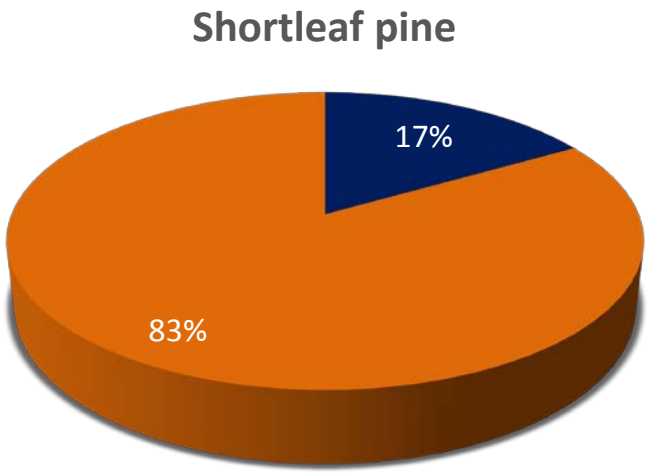

Species tested

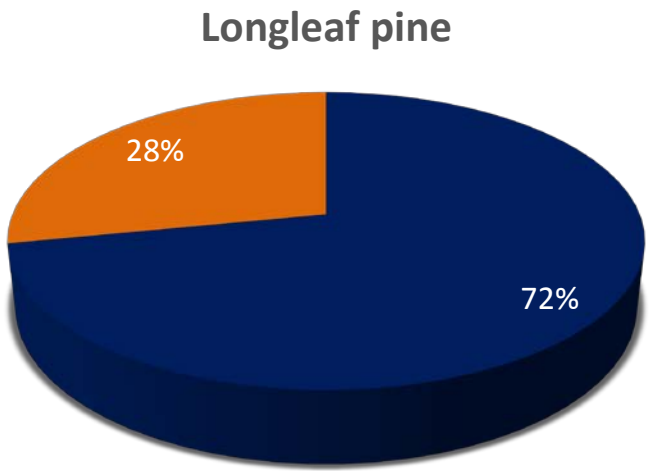
Species	Families / Seed lots Collections / Sources	Members / Cooperators
Longleaf Pine	49	5
Loblolly Pine	29	4
Shortleaf Pine	24	4
Slash Pine	22	4
White Pine	8	3
Pitch Pine	6	2
Virginia Pine	4	2
Sand Pine	1	1

Results of seed screening







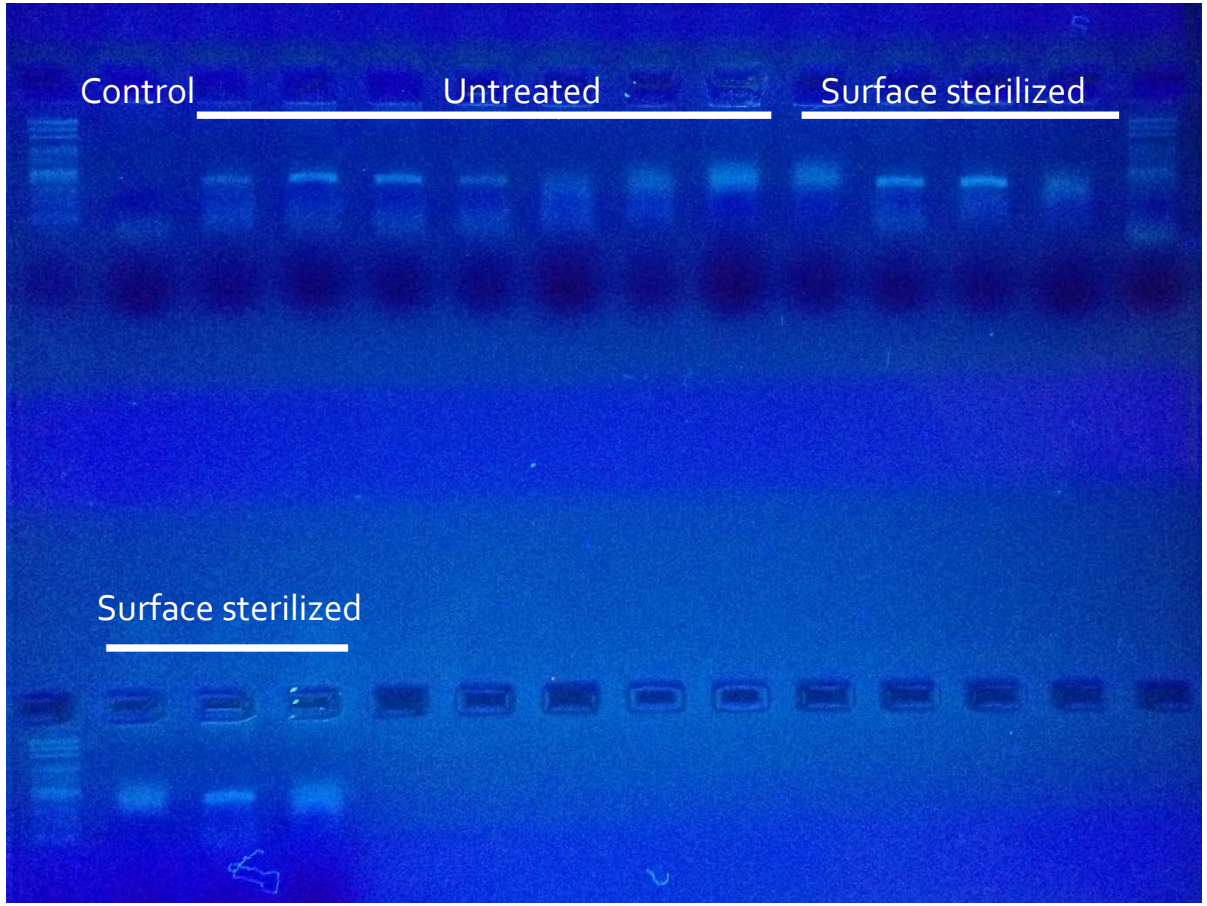


% Seed lots infected

% Seed lots un-infected

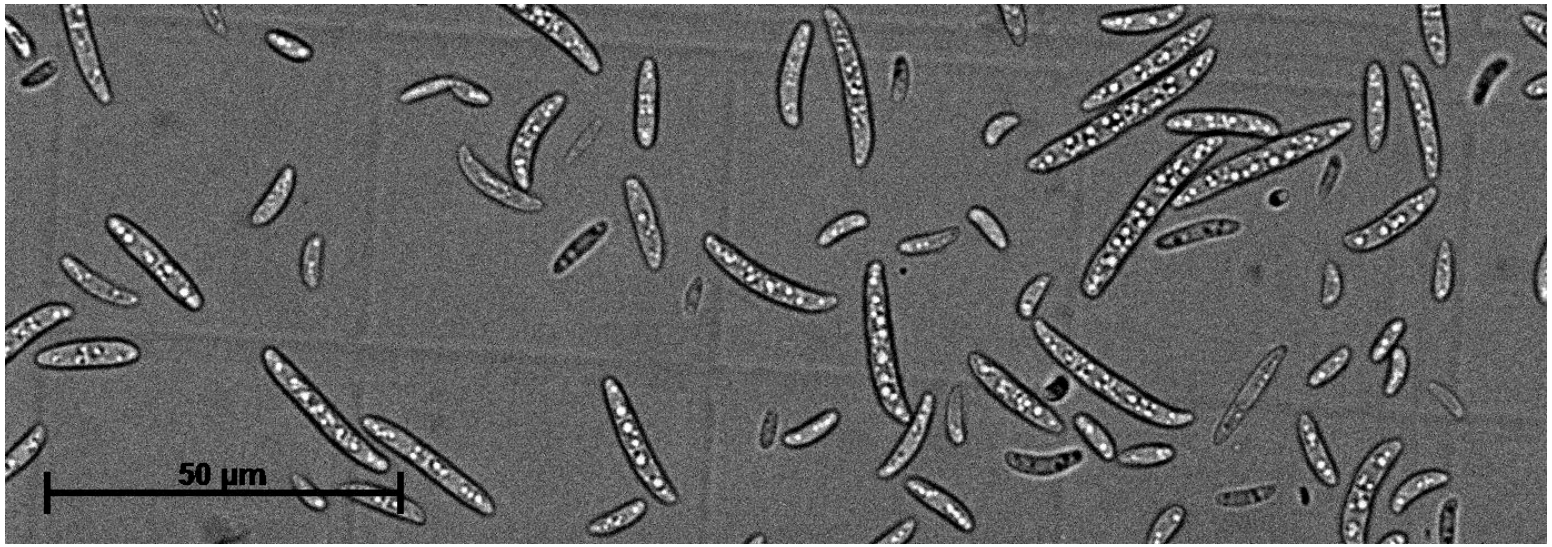
Surface sterilization results

- Seed was screened for the presence of *F. circinatum* following surface sterilization of seed lots



PCR detection limit

- Seed lots representing several pine species were sterilized
- A sub-sample of autoclaved seed were infected with a single isolate of *F. circinatum*
- Varying quantities of infected seed, ranging from 1 – 13 seed per sample of 400 seed were screened for the presence of *F. circinatum* for each pine species



Detection limits of method

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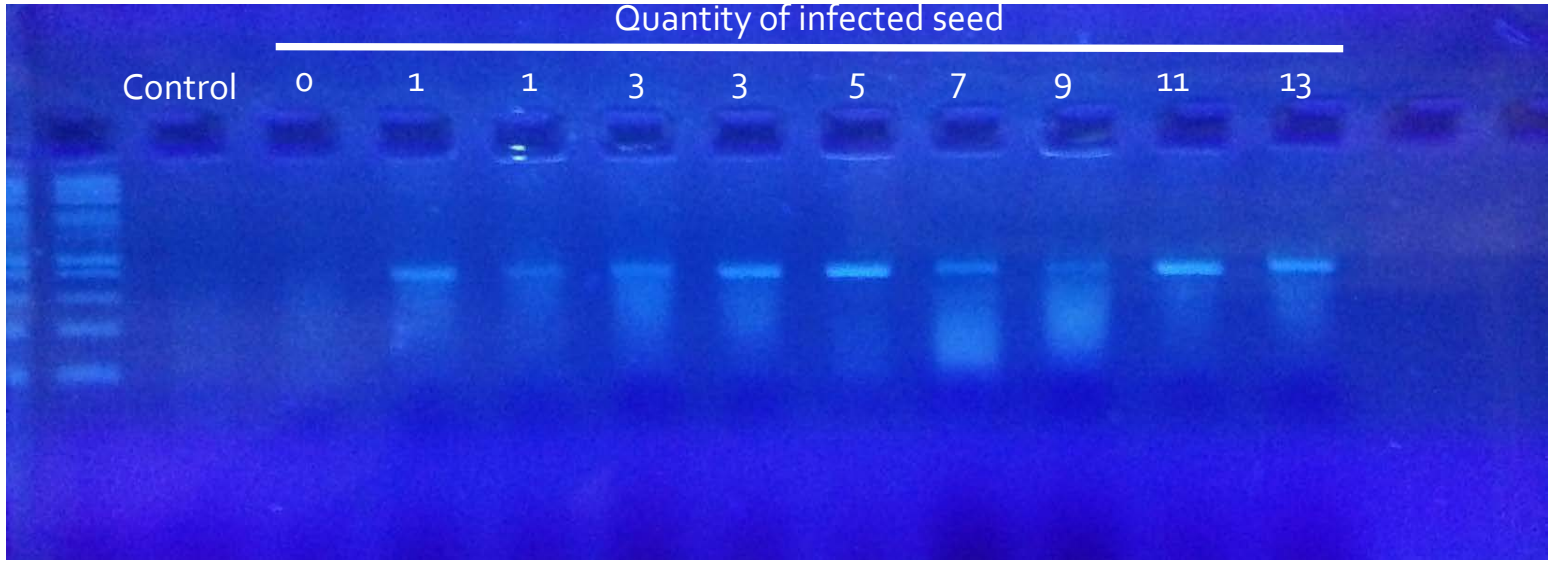
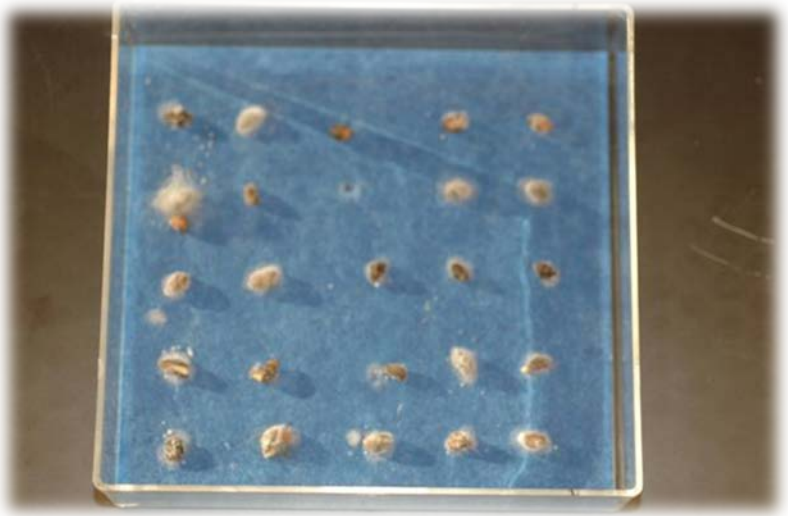




Way forward

- To actually quantify seed infestation levels of seedlot or seedling samples screened, we propose to undertake research to optimize and improve the sensitivity and precision of our developed method by using qPCR.

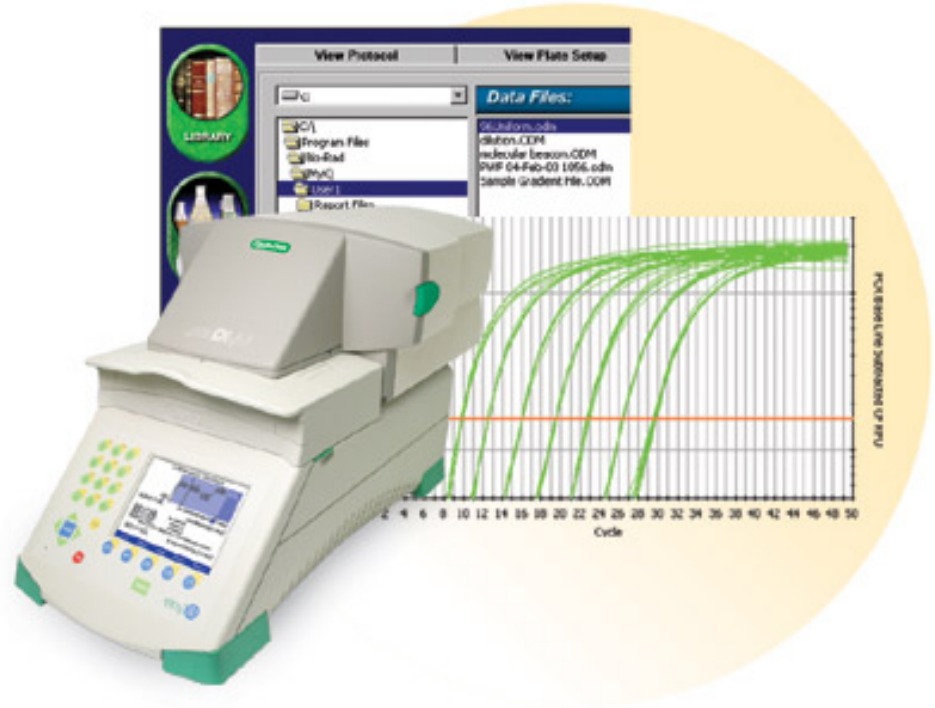
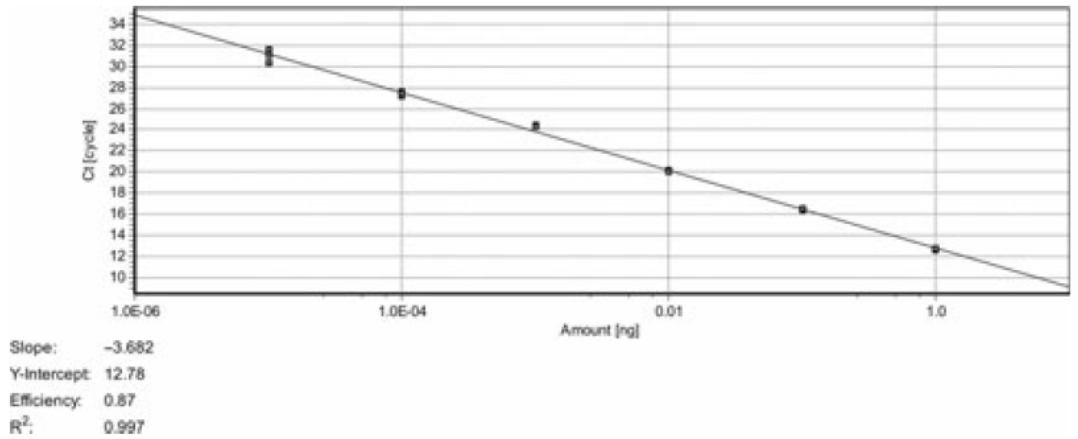


Image: http://www.gene-quantification.de



Dreaden et al. (2012) Forest Pathology, **42,**

Screening services

- We have submitted our protocol to the International Seed Testing Authority for consideration as an official method. Our intention is that once the molecular method is approved by the ISTA, that it be used to rapidly screen pine seed lots to assist in the reduction of Pitch Canker spread.
- Although this method has not as yet been approved by the ISTA, we have decided to begin seedlot and/or seedling screening service to our members. Listed below are some points that cover most of the information you need to know.
  - One sample = 400 seed from a single seedlot. Or 1 seedling.
- Multiple samples can be shipped together as long as they are clearly identified.
- A "Pitch Canker Sample Submission Form" should accompany each sample. This form can be found on the "Members Only" section of the Nursery Cooperative web site.