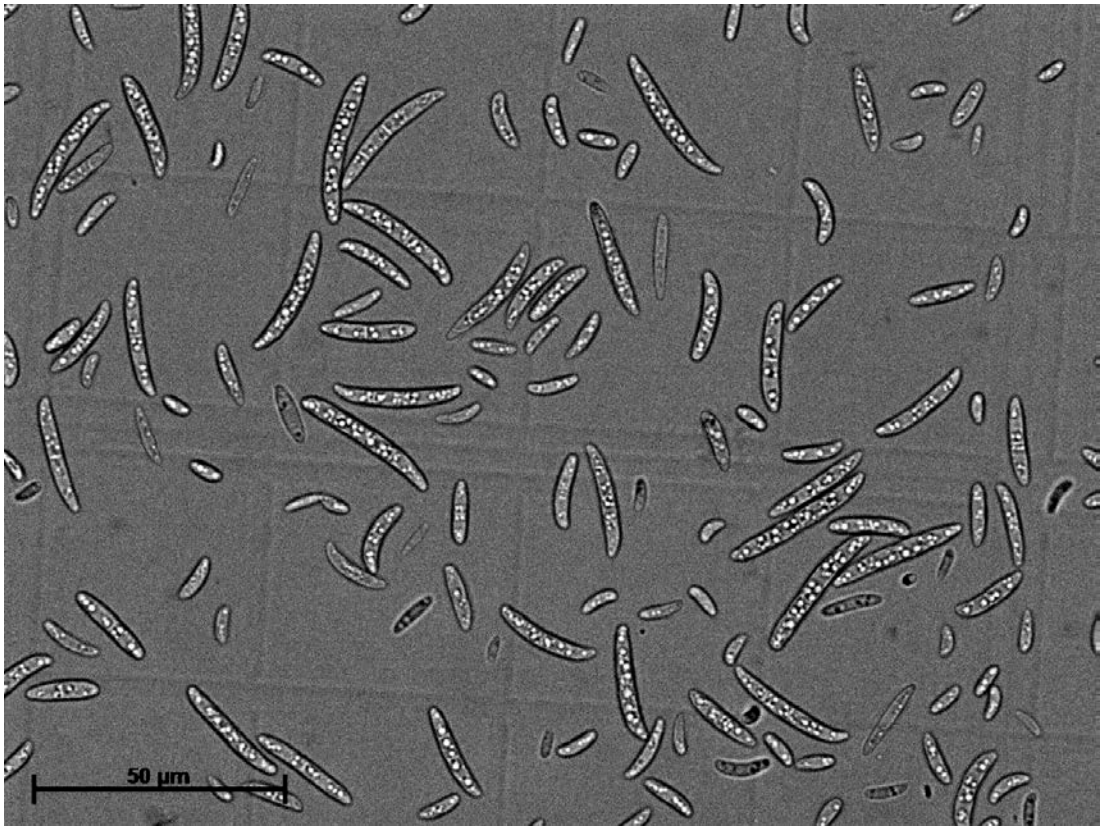
Rapid Identification Test for Pitch Canker



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Fusarium circinatum

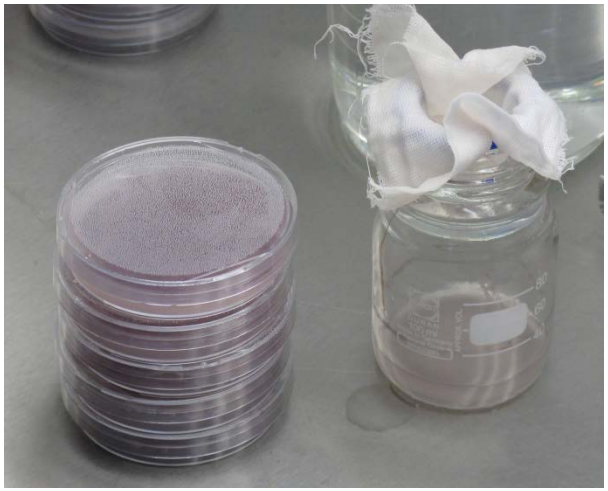
- Pitch canker is caused by the fungal pathogen *Fusarium circinatum* (*Gibberella circinata*), a serious disease that affects numerous pine species grown Internationally
- The term pitch canker refers to the large resinous cankers that develop on roots, trunks, branches and reproductive organs of mature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

Fusarium circinatum

- Studies have shown that the fungus probably originated in Mexico or Central America and was accidentally introduced into pine-growing regions around the world
- *Fusarium circinatum* internationally threatens plantation forestry that rely on susceptible pine species such as monterey pine, mexican weeping pine and longleaf pine
- Accidental introduction of *F. circinatum* into California, Asia, Europe, South Africa and South America

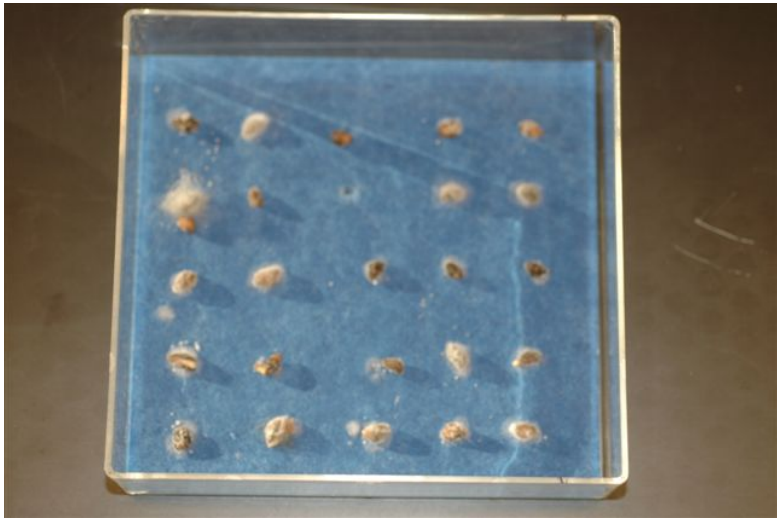
Seed certification

- *Fusarium circinatum* is readily transported in and on pine seeds and cuttings
- 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 Centre to screen for the pathogen



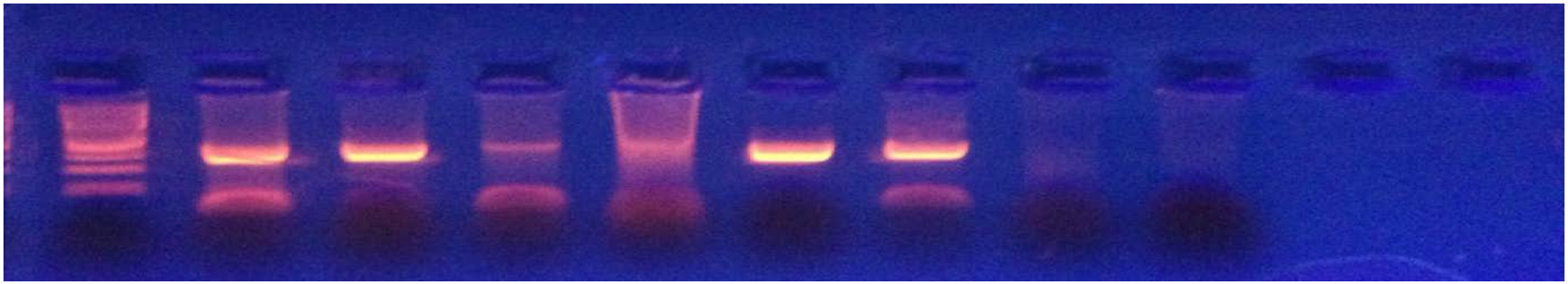
Seed screening method

- 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 method



DNA extraction and PCR 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

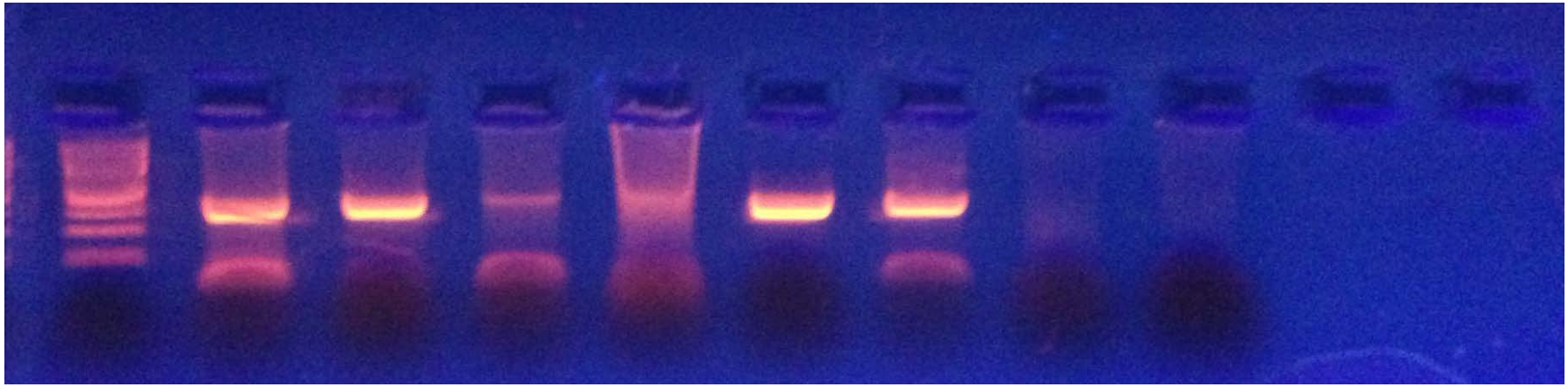


DNA extraction and PCR procedure

- 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

Results

- To date species specific primers have been developed by our collaborators at the University of Florida
- Numerous seed lots, representing several of the most commonly planted pine species in the southeastern US were sourced and screened

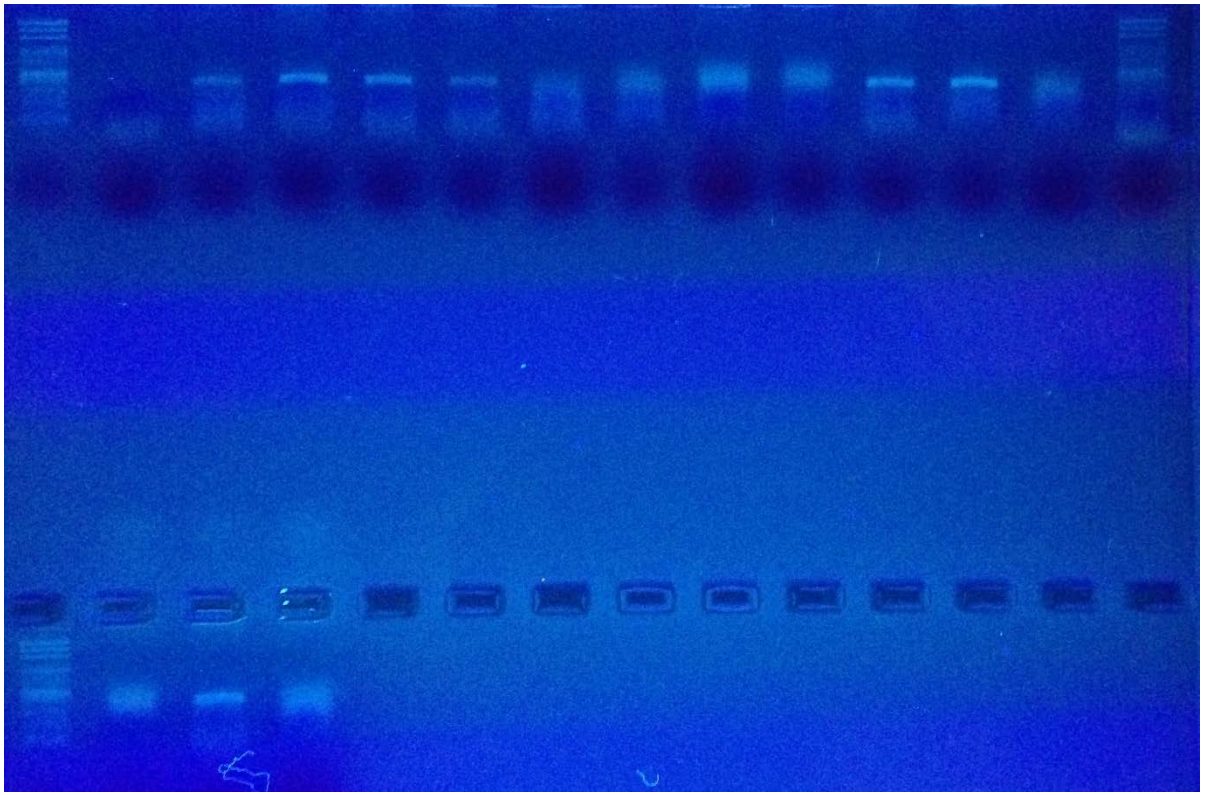


Species and collections to date:

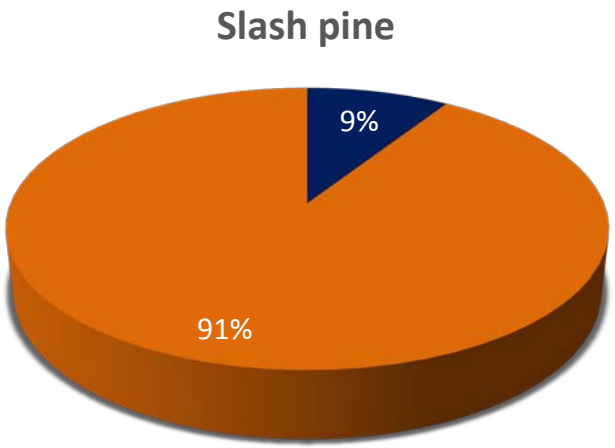
Species	Families / Seed lots Collections / Sources	Members / Cooperators
Longleaf Pine	49	5
Loblolly Pine	29	4
Shortleaf Pine	24	4
Slash Pine	22	4
White Pine	8	3
Pitch Pine	6	2
Virginia Pine	4	2
Sand Pine	1	1

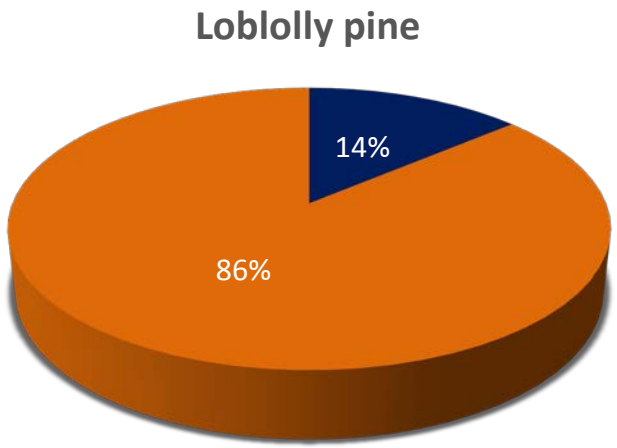
Results

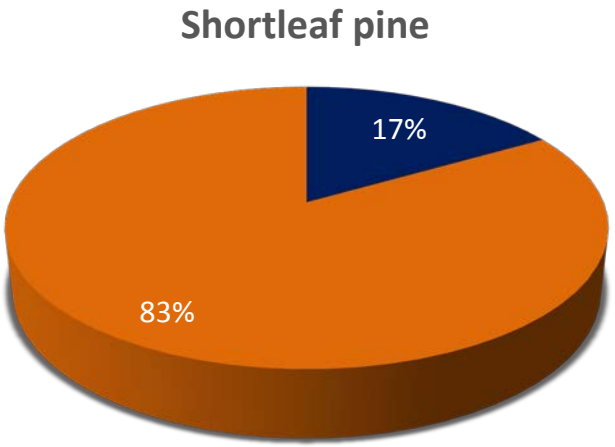
- DNA extraction and PCR amplification protocols have been developed and refined
- Contact has been made with ISTA regarding steps to be undertaken for protocol approval

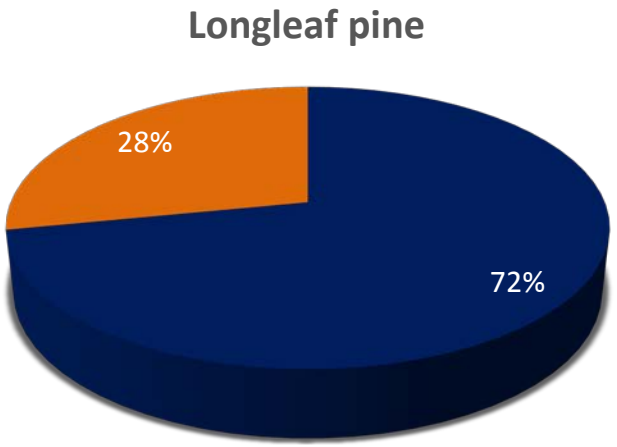


Results





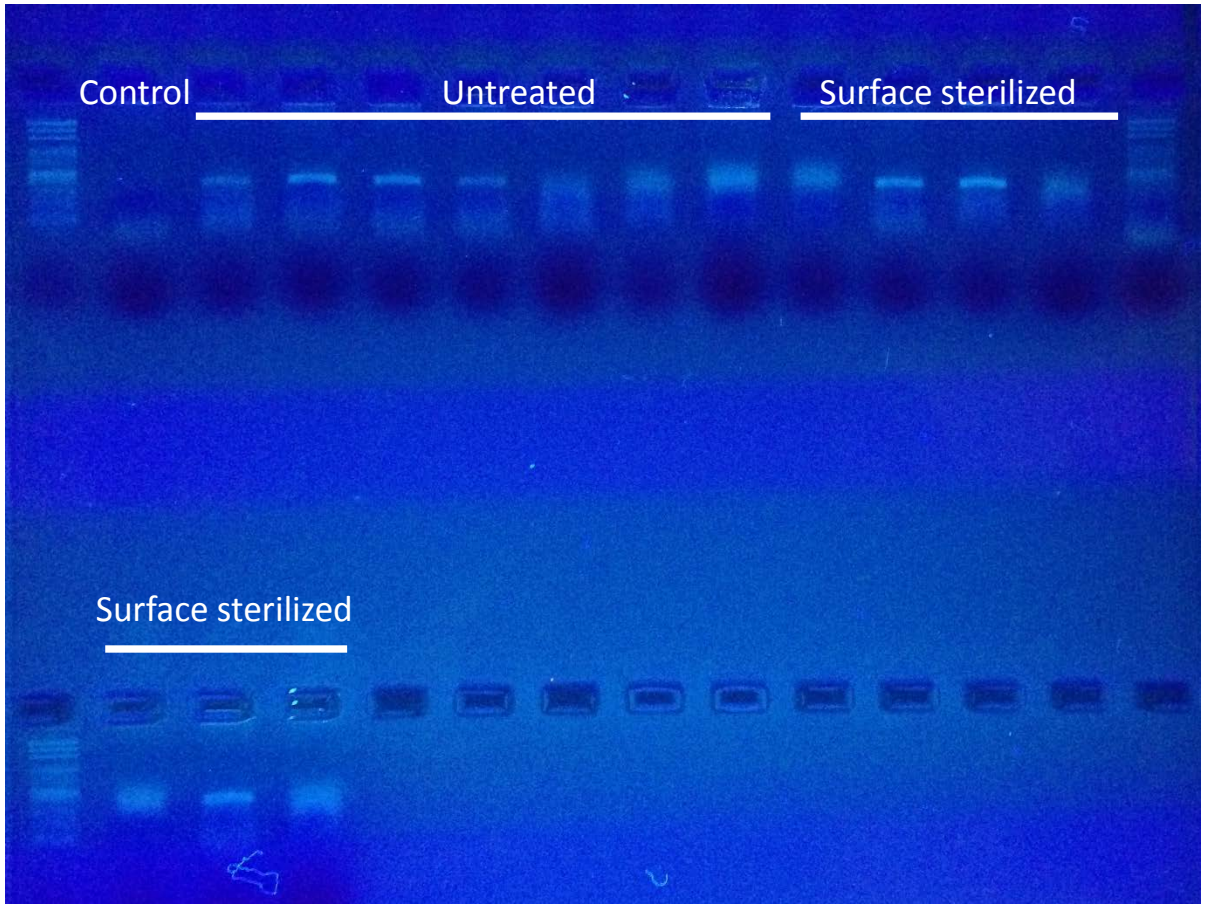




% Seed lot infected % Seed lot un-infected

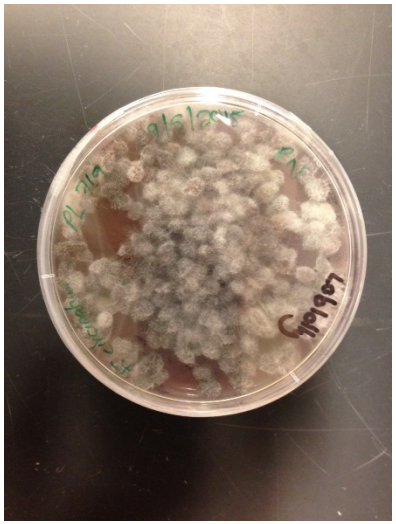
Surface sterilization results

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

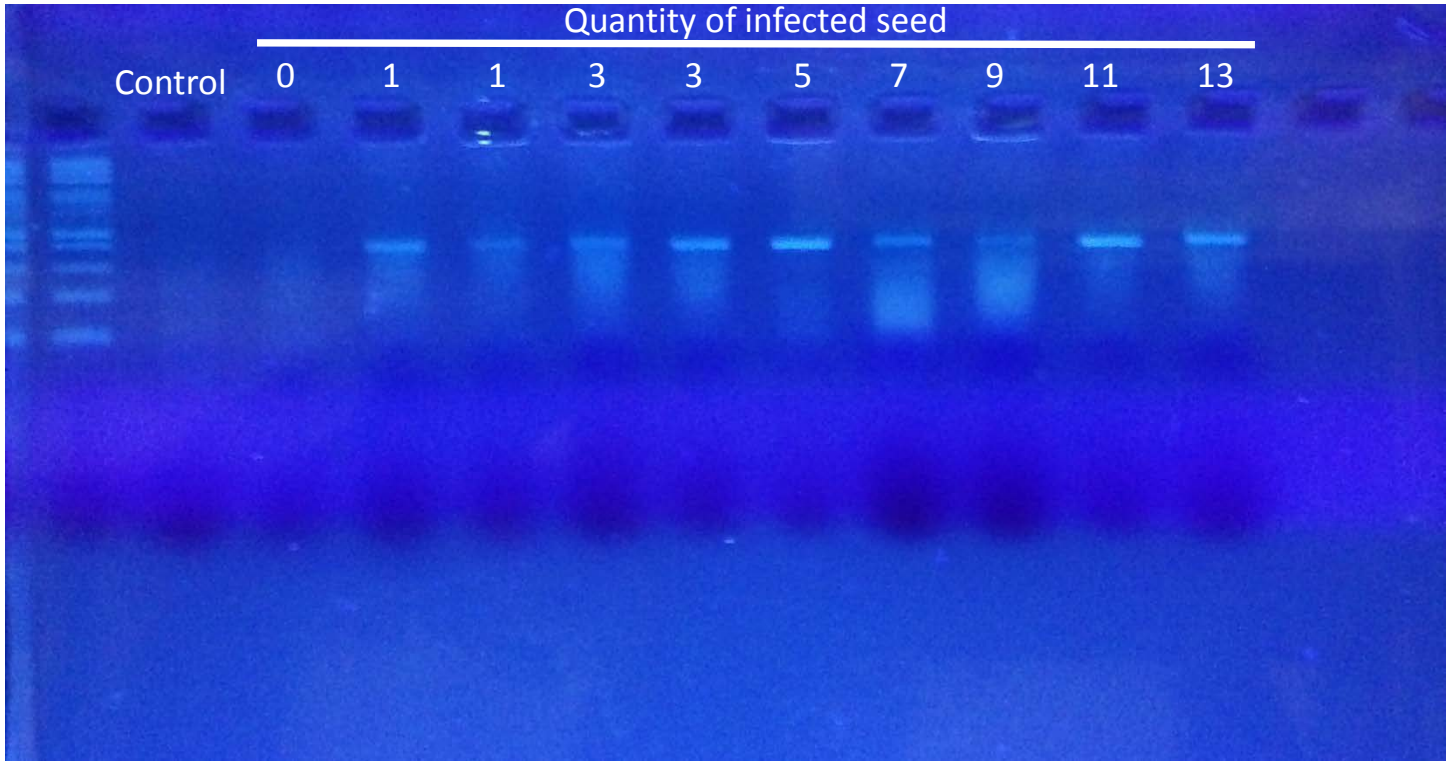


PCR detection limit

- 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

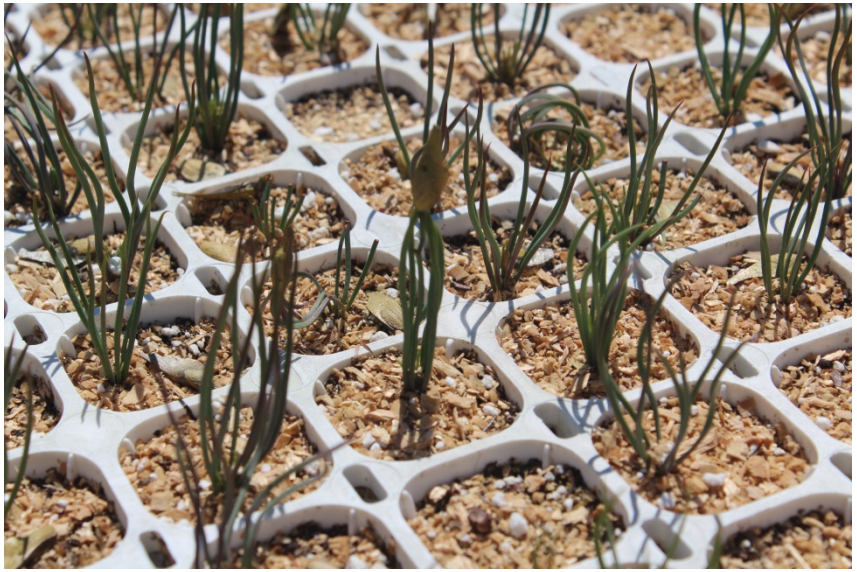



PCR detection limit



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



Acknowledgements

- Forest Health Dynamics Laboratory

SOUTHERN FOREST NURSERY MANAGEMENT COOPERATIVE

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FLORIDA FOREST SERVICE Agriculture & Consumer Services

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