Pitch canker as a model to discover biological drivers of disease in a changing climate

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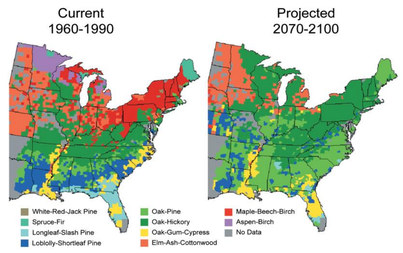
Introduction

Climate change can affect forest health at multiple levels

- Natural and agricultural ecosystems affected by climate change; includes new diseases occupying areas where they previously didn't exist.
- Disruption of disease triangle as current host-pathogen balance is affected.
- Modeling the distribution of predicted fungal isolates under future conditions could help mitigate the impact of potential pitch canker outbreaks



Current and projected shifts on forest ecosystems in the Eastern United States, from Karl et al., 2009 [1].

Pitch canker is one of the main diseases in pine

- Caused by the fungus *Fusarium circinatum* [2].
- Outbreaks favored by high temperature and humidity [3], predicted factors in future climate.
- Host resistance to pitch canker is quantitative and heritable [4,5]
- Current mitigation strategies rely mainly on breeding and selection of resistant host material.

Loblolly pine rooted cutting showing symptoms of pitch canker at 8 weeks after inoculation with *F. circinatum* microconidia.

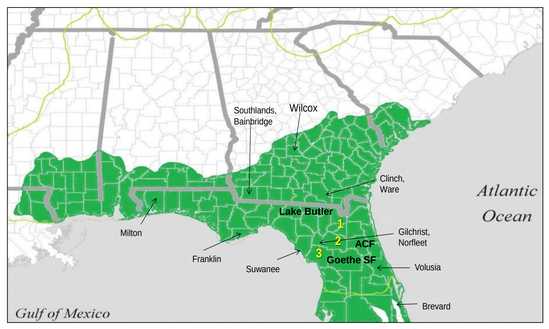
Specific Aims:

1) **Evaluate differential responses of *F. circinatum* isolates to abiotic stress**
2) **Understand the phenology and infectivity of *F. circinatum* in north-central Florida**
3) **Develop epidemiological models to predict future pitch canker outbreaks**

Methods

Fungal isolates from Florida and Georgia

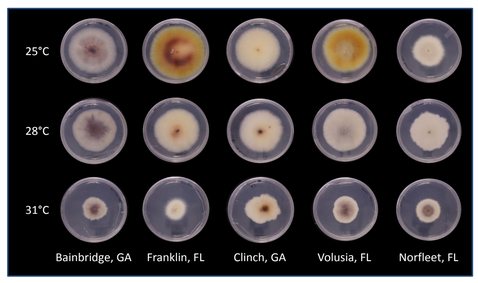
- Samples obtained from live tissue and from stored isolates
- Site coordinates recorded
- Culture in Acid Potato Dextrose Agar medium
- Single spore isolation for inoculation
- Transfer to filter paper for storage and transportation



Map of southeastern United States showing the natural range for slash (dark green) and loblolly (light green) pines and the location of *Fusarium circinatum* isolates used in this study. Yellow numbers are field locations used for spore collection and placement of weather stations: 1) Lake Butler, 2) Austin Cary Forest, 3) Goethe State Forest.

Growth and sporulation

- Split-plot design: 3 replicates, 3 treatments
- Temperatures: 25°C, 28°C, and 31°C
- Diameter of cultured mycelium recorded daily for eight days
- Spore count analysis at two weeks post-inoculation
- Spore germination count after 24 hours in sterile water



Fusarium circinatum mycelium cultures at three temperatures: 25, 28, and 31 ºC. Photo: T. Quesada.

Spore traps and weather stations

- 3 sites: Lake Butler, Austin Cary Forest, and Goethe State Forest
- 6 traps and 1 weather station per site
- Weekly trap switch; weather records every 15 minutes
- DNA extractions using modified CTAB method
- Quantitative PCR using *F.circinatum*- specific primers CIRC1L and CIRC4L [6]

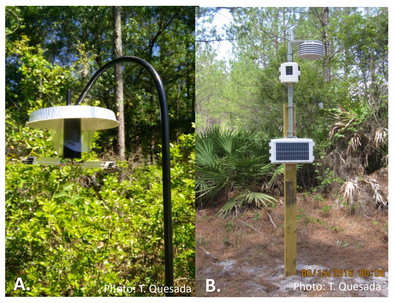
Predictive modeling

- Historical data on slash and loblolly pine measurements
- 112, 919 trees at four Florida sites
- Predictor variables: disease presence/absence, tree height, tree age, DBH, average temperature (monthly min., monthly max), average rainfall
- Predictive model generated using random forest classifier to identify best predictors for presence of pitch canker

Pathogenicity tests

- 8 fungal isolates, 3 replicates,
- 20 seedlings/replicate
- 1 resistant loblolly and 2 susceptible slash pine families
- Inoculum density: 100,000 spores/ml
- Lesion length and survival recorded at 12 weeks after inoculation.

Pine seedlings at four weeks after inoculation showing initial symptoms of pitch canker disease. Photo: Sunny Lucas, USDA-RSC



A. In-house spore trap made with a garden motor and vaseline-covered microscope slides**. B.** Weather station placed on each site.

Numerical independent variables measured in the study. The data is aggregated across all four sites.

Variable	Median	SD
Pitch canker (presence (1)/absence(0))	NA	NA
Total tree height (ft)	35.5	61.6
Tree age at inventory (years)	6.7	3.4
Diameter at breast height (DBH) (inches)	5.8	8.6
Species (loblolly or slash)	NA	NA
Annual average monthly minimum temperature(°C)	13.7	1.06
Annual average monthly maximum temperature (°C)	26.8	0.77
Annual average rainfall total (mm)	1251.1	276.5

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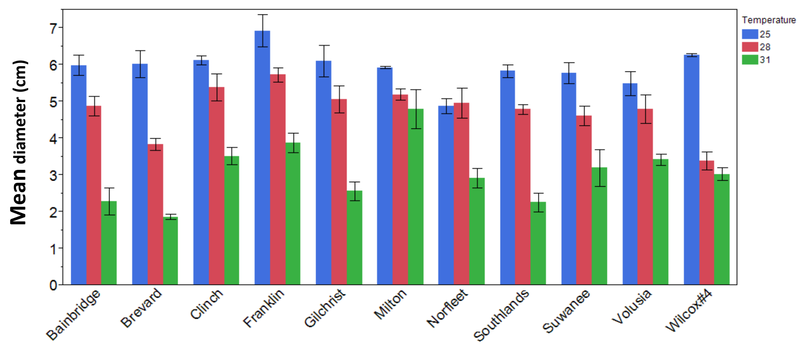
Results

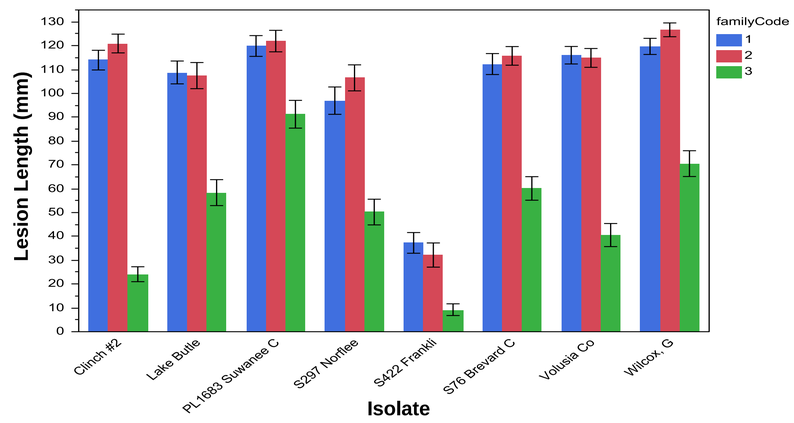
Significant differences in growth were observed among isolates from different geographical sites and temperatures



Effect	P-value
Rep	0.2578
Incubator	0.5971
Temperature	**<0.0001**
Isolate	**<0.0001**
Temp.*Isolate	**0.0021**

Least-square means for growth of *Fusarium circinatum* isolates after 8 days in culture at 25, 28, and 31 °C. P-values for fixed effects are shown in the side table. Significant effects (P < 0.05) are shown in bold.

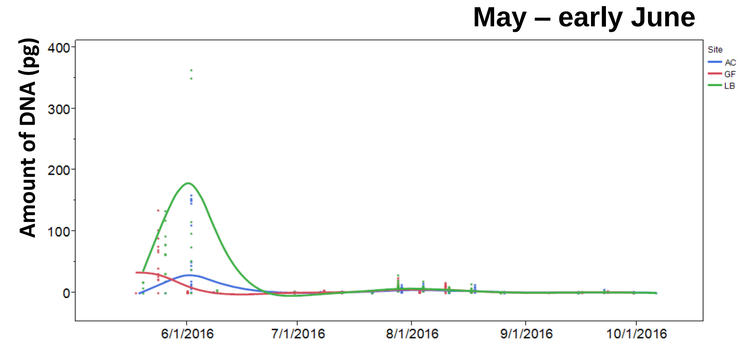
Isolates showed significant differences in pathogenicity in three host open-pollinated loblolly and slash families



Effect	P-value
Rep	0.1632
Isolate	**<.0001**
Host	**<.0001**
Rep*Isolate	**0.007**
Rep*Host	0.2631
Isolate*Host	**<.0001**
Rep*Isolate*Host	0.721

Least-square means for *F. circinatum* lesion length at 12 weeks post-inoculation with 8 different isolates. P-values for fixed effects are shown in the side table. Significant effects (P < 0.05) are shown in bold. Family codes: 1 (blue) and 2 (red) – Susceptile slash pine families; 3 (green) – Resistant loblolly pine family.

Quantitative PCR showed a peak of high spore abundance in late May – early June



Mean amount (pg) of *F. circinatum* DNA amplified using qPCR with species-specific primers. Samples were collected weekly from spore traps located at Austin Cary Forest (AC), Goethe State Forest (GF), and Lake Butler (LB) sites.

Predictive modeling on historical data show a 91% skill at identifying presence of pitch canker given the selected predictors

All-in-one confusion matrix, and classification accuracy for model and cross validation. The predictors selected based on a random forest classifier were: age at inventory, diameter at breast height, and species, followed by annual average maximum temperature, total tree height, and annual average total rainfall.

	Confusion matrix				Classification accuracy for model		Classification accuracy for cross-validation	
	Absence	Presence	class.error		Absence	Presence	Absence	Presence
Absence	386	61	13.6%	**users accuracy**	86.3%	90.3%	86.7%	91.1%
Presence	43	404	9.6%	**producers accuracy**	89.8%	86.9%	90.2%	87.0%

Overall model accuracy: 94.18
OOB estimate of error rate: 11.63%

Ongoing Work

- Add isolates from more northern areas within loblolly pine geographical range.
- Repeat spore collections and pathogenicity tests
- Use of geographical, biological, and environmental information to validate epidemiological models for predicting future pitch canker outbreaks.

References

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