Monitoring cotton soil-borne seedling diseases through metabarcoding

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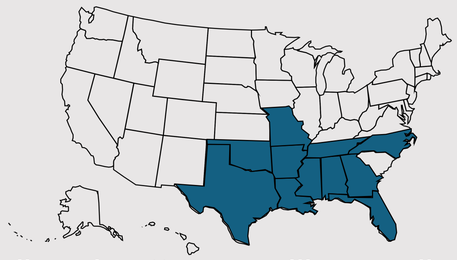
BACKGROUND

- Seed and soilborne fungal pathogens like *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* negatively impact cotton production across the south
- Seedling diseases can cause loss of stand and uniformity requiring replanting, increased input cost, delayed crop development, and reduced yield
- There is a lack of information available that combines environmental, soil classes, pathogen populations, and disease outcomes

OBJECTIVES

1. Determine pathogen abundance across cotton belt in 2023 and 2024
2. Identify regional patterns in pathogen abundance in 2023 and 2024

METHODS



Soil and cotton seedlings collected from National Cotton Seed Treatment (NCST) plots across southeastern USA in 2023 + 2024

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Extract DNA
(bulk soil + rhizosphere + root tissue)

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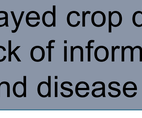
Primers:
Fungal
ITS1F
ITS4
Oomycete
ITS6
ITS7



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Amplicon sequencing on Illumina MiSeq

- Environmental variables: NASA Power
- Soil variables: USDA Web Soil Survey
- Seedling percent stand used as measure of disease pressure
- **Fungal sequence data filtered to only include taxa from genera *Fusarium*, *Rhizoctonia*, and *Berkeleyomyces***

RESULTS

- *Fusarium oxysporum* and *Fusarium solani* sp. complex found at all sites (Fig. 1)
- *Rhizoctonia solani* found at all sites except one (MS-Verona; Fig. 2)
- *Berkeleyomyces rouxiae* (former *Thielaviopsis basicola*) found at five out of 17 sites (Fig. 3)
- *Globisporangium irregulare* found at all sites (Fig. 4)
- Fungal richness decreases with latitude and seedling percent stand, and is lowest in root tissue compared to bulk soil and rhizosphere, respectively (Fig. 5)
- Fungal evenness decreases with percent organic matter and seedling percent stand, and is lowest in root tissue compared to rhizosphere and bulk soil, respectively (Fig. 6)
- Fungal composition influenced by longitude, latitude, percent sand, seedling percent stand, percent organic matter, and mean rain 3DPP (Fig. 7)
- Oomycete richness increased with latitude, longitude, mean specific humidity 3 days post planting (3DPP) but decreased with percent sand and mean surface temperature 3DPP, and is lowest in root tissue compared to bulk soil and rhizosphere (Fig. 8)
- Oomycete evenness decreased with seedling percent stand but increased with longitude, and is lowest in root tissue compared to bulk soil and rhizosphere, respectively (Fig. 9)
- Oomycete composition influenced by latitude, longitude, percent sand, mean rain 3DPP, mean surface temperature 3DPP, seedling percent stand and percent clay (Fig. 10)

FUTURE WORK

- Incorporation of functional gene content data to determine functional potential and identify regional patterns
- Input fungal and oomycete community data plus functional gene content into Random Forest model to assess if we can predict seedling percent stand or other measures of plant health

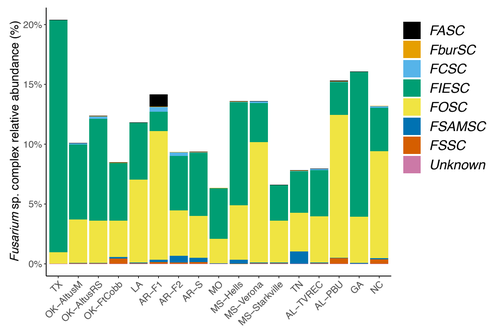


Fig 1. Relative abundance of Fusarium sp. complexes by sampling site. FASC = *Fusarium albidum* sp. complex; FburSC = *Fusarium burgessii* sp. complex; FCSC = *Fusarium chlamydosporum* sp. complex; FIESC = *Fusarium incaarnatum-equiseti* sp. complex; FOSC = *Fusarium oxysporum* sp. complex; FSAMSC = *Fusarium sambucinum* sp. complex; FSSC = *Fusarium solani* sp. complex.

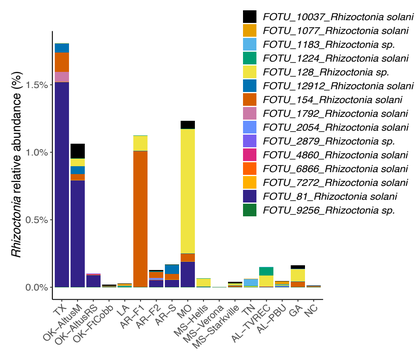


Fig 2. Relative abundance of *Rhizoctonia* by sampling site.

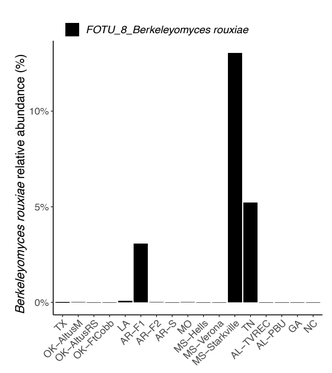


Fig 3. Relative abundance of *Berkeleyomyces rouxiae* (former *Thielaviopsis basicola*) by sampling site.

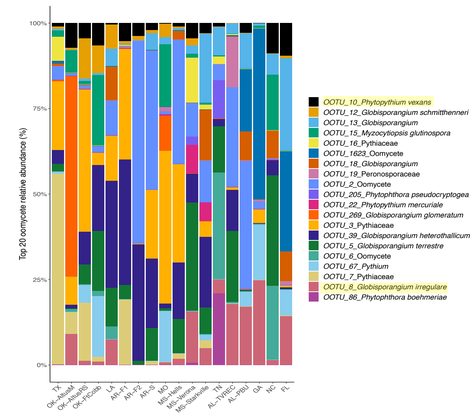


Fig 4. Relative abundance of the top 20 oomycete species present by sampling site. Highlighted taxa indicate known cotton pathogen.

Fungi

includes only genera *Fusarium*, *Rhizoctonia*, and *Berkeleyomyces

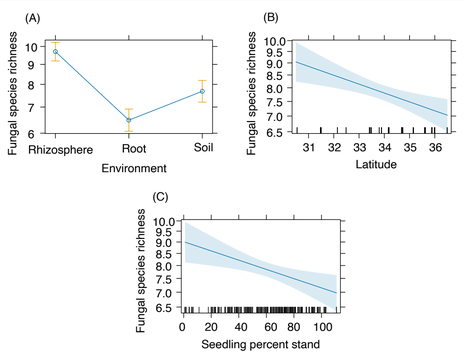


Fig. 5 Generalized linear model with Poisson distribution showing the effect of (A) environment, (B) latitude, and (C) seedling percent stand on fungal species richness, where the fitted line represents the estimated relationship between the variables.

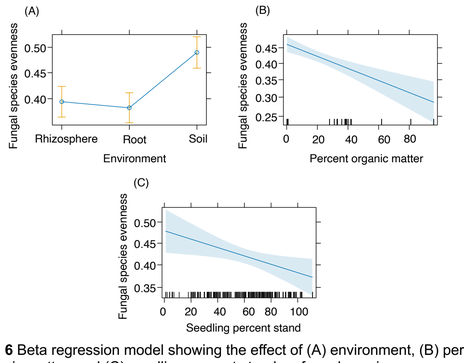
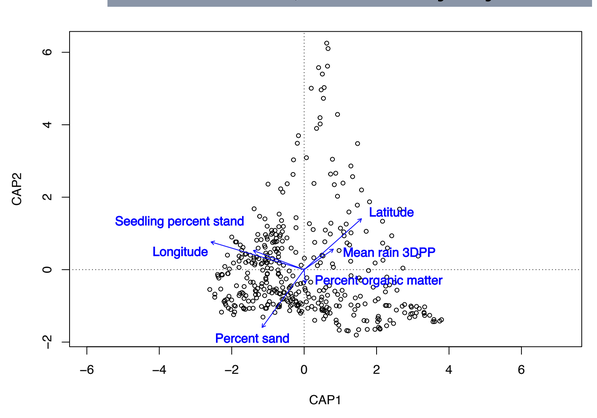


Fig. 6 Beta regression model showing the effect of (A) environment, (B) percent organic matter, and (C) seedling percent stand on fungal species evenness, where the fitted line represents the estimated relationship between the variables.



	R2.adj	Df	AIC	F	Pr(>F)
Longitude	0.031404	1	1879	11.4984	0.002 **
Latitude	0.040712	1	1877	4.0126	0.002 **
Percent sand	0.049818	1	1875	3.9577	0.002 **
Seedling percent stand	0.057404	1	1873.5	3.4447	0.004 **
Percent organic matter	0.062543	1	1872.9	2.6043	0.006 **
Mean rain 3DPP	0.066317	1	1872.7	2.1334	0.024 *
<All variables>	0.068417				

Fig. 7 Canonical Analysis of Principal Coordinates (CAPSCALE; top) shows the relationship between fungal community composition (species relative abundance) and significant explanatory variables, with each point representing a sample (n = 393) and each vector indicating the direction and strength of the explanatory variable's influence. The axes represent the primary gradients of variation. The table (below) contains results from the ANOVA test ran after bidirectional model selection to identify the most important variables for explaining variation in the ordination.

Oomycetes

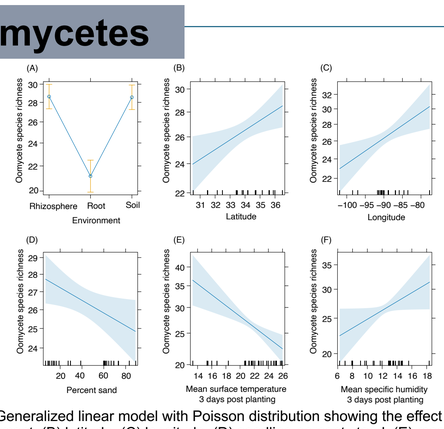


Fig. 8 Generalized linear model with Poisson distribution showing the effect of (A) environment, (B) latitude, (C) longitude, (D) seedling percent stand, (E) mean surface temperature three days post planting, and (F) mean specific humidity three days post planting on oomycete species richness, where the fitted line represents the estimated relationship between the variables.

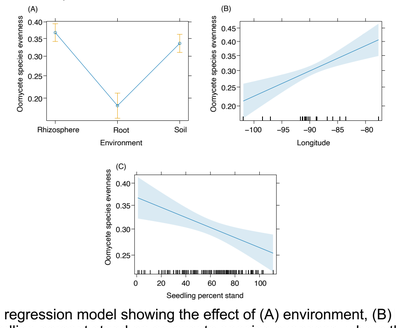
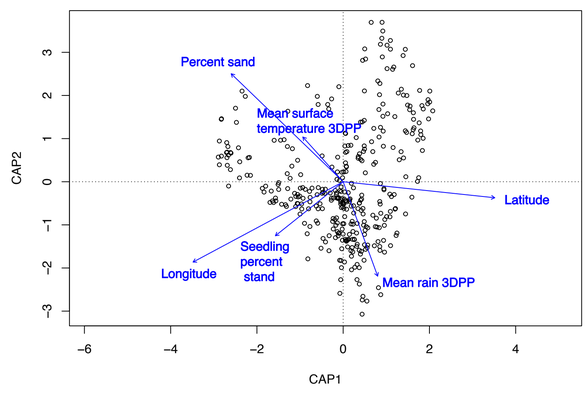


Fig. 9 Beta regression model showing the effect of (A) environment, (B) longitude, and (C) seedling percent stand on oomycete species evenness, where the fitted line represents the estimated relationship between the variables.



	R2.adj	Df	AIC	F	Pr(>F)
Longitude	0.029484	1	1885.6	10.6557	0.002 **
Latitude	0.048816	1	1880.2	7.3832	0.002 **
Percent sand	0.067637	1	1874.9	7.3015	0.002 **
Seedling percent stand	0.080846	1	1871.4	5.4272	0.002 **
Mean rain 3DPP	0.091145	1	1868.9	4.4474	0.002 **
Mean surface temperature 3DPP	0.099373	1	1867.1	3.7417	0.002 **
<All variables>	0.103149				

Fig. 10 Canonical Analysis of Principal Coordinates (CAPSCALE; top) shows the relationship between oomycete community composition (species relative abundance) and significant explanatory variables, with each point representing a sample (n = 393) and each vector indicating the direction and strength of the explanatory variable's influence. The axes represent the primary gradients of variation. The table (below) contains results from the ANOVA test ran after bidirectional model selection to identify the most important variables for explaining variation in the ordination.

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Sources:

1. DeVay, J. E., and C.S. Rothrock. 2001. Control of seedling diseases. In: T.L. Kirkpatrick, and C. S. Rothrock, editors, Compendium of cotton diseases. American Phytopathological Society, St. Paul, MN. pp. 19-20.
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