

# PCR, Detection Times Compared to ELISA

## National Soybean Rust Symposium 2005—Nashville, TN

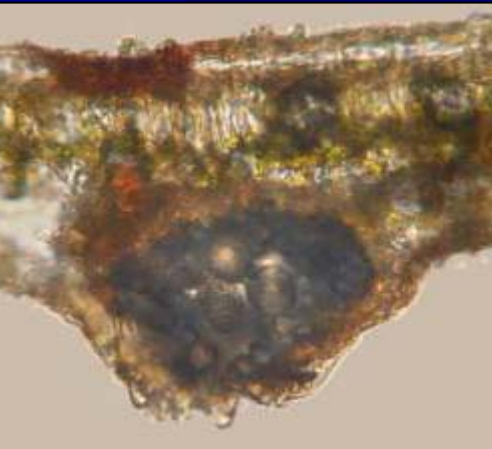
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# Acknowledgements

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- **University of Kentucky**
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- **North Carolina State University**
  - Tom Creswell
- **University of Tennessee**
  - Kurt Lamour

# Conclusions

## Time Requirements (per sample)

- Average time required per sample was approximately the same for ELISA and real-time PCR (34 min).
- For traditional PCR the average time per sample was 11 min.
- Average time for visual observation of positive samples was 4 min and for negative was 8 min.

# Conclusions

## Time (per group of samples)

- Average time required per ELISA run was 170 min, real-time PCR was 220 min, traditional PCR was 317 min.
- For ELISA, time per run was strongly (80%) correlated to number of samples per run.
- For real-time PCR, time per run was weakly (34%) correlated to samples per run.

# Conclusions

## Agreement between protocols

- ELISA plate test agreed with visual observation 100% and with standard PCR 100% (when used)
- ELISA and real-time PCR methods agreed 89% of the time.

# Conclusions

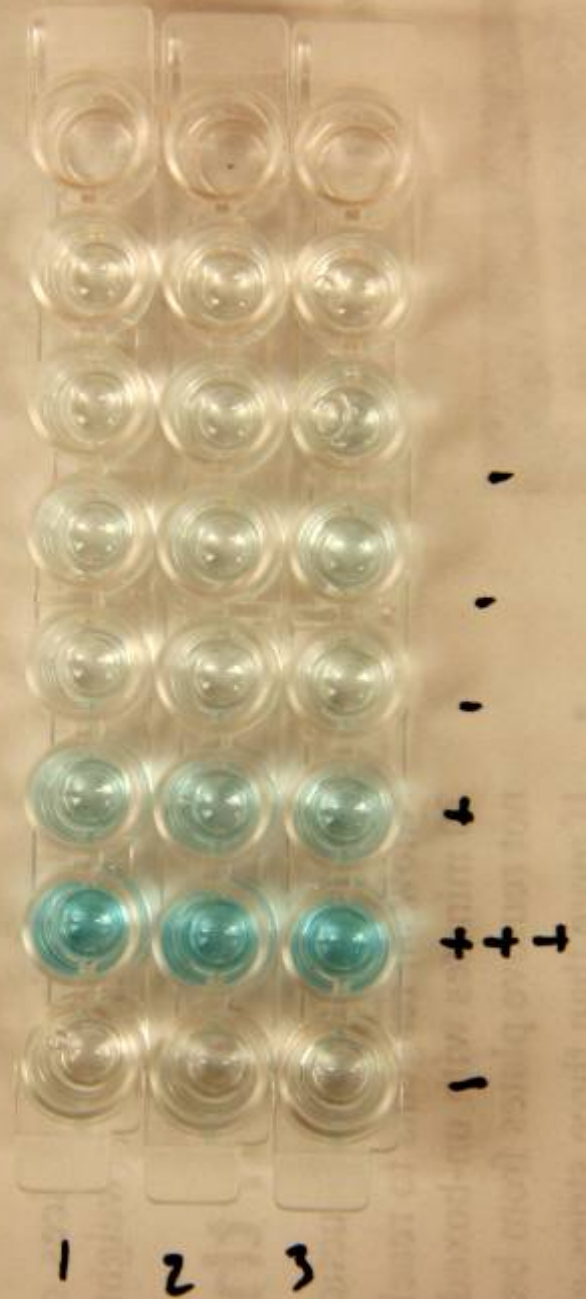
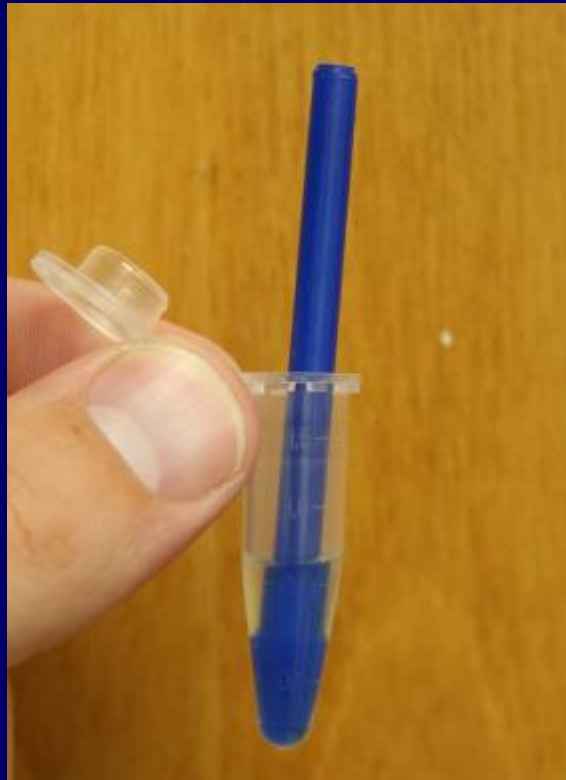
## Summary

- With proper training, diagnosticians are proficient at diagnosing SBR after observation of pustules and spores through a dissecting microscope.
- ELISA was found to be as good as visual observation, but took about 8 times longer per sample on average.
- PCR is the only way to distinguish SBR pathogens. Our data suggest that real-time PCR assays could be sensitive to latent infection or capricious spore deposition—not the objective of the study.

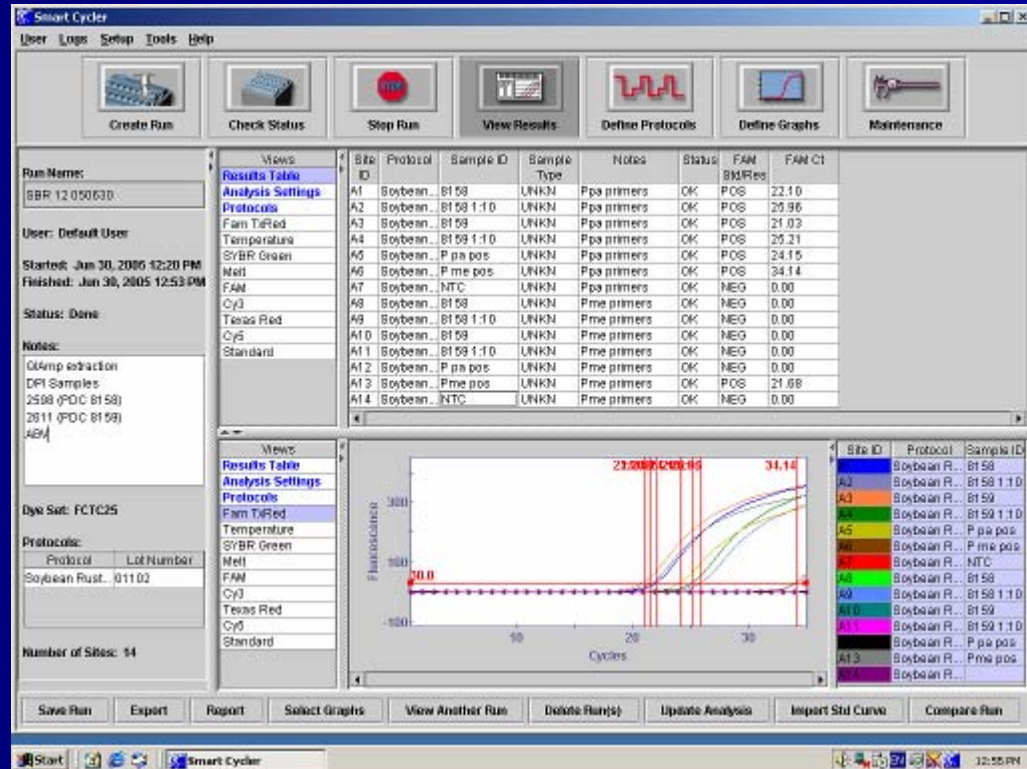
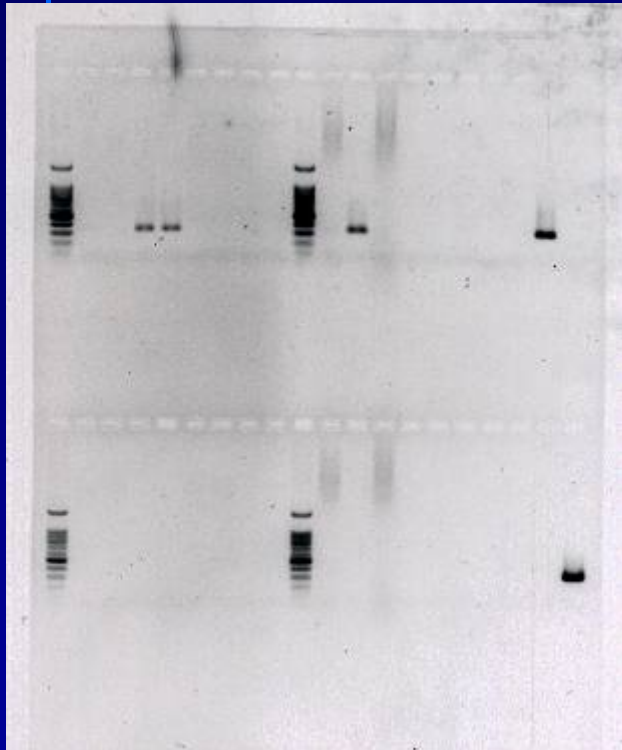
# Visual observation



# ELISA



# PCR



# Real-Time Discrepancies

Real-time PCR did not agree with the other methods for 11% of the samples (pos + neg)

- One replicate of one sample was a false negative due to user error (not enough template)—only false negative
- 15 Samples (5 in triplicate) returned positive for *P. meibomia*. No pustules, spores, sent on to USDA
- Six presumed negative replicates returned “weak” positive from three samples all at UF:

Ct: SBR2	31	31	32
SBR5	34	-	-
SBR9	33	35	-

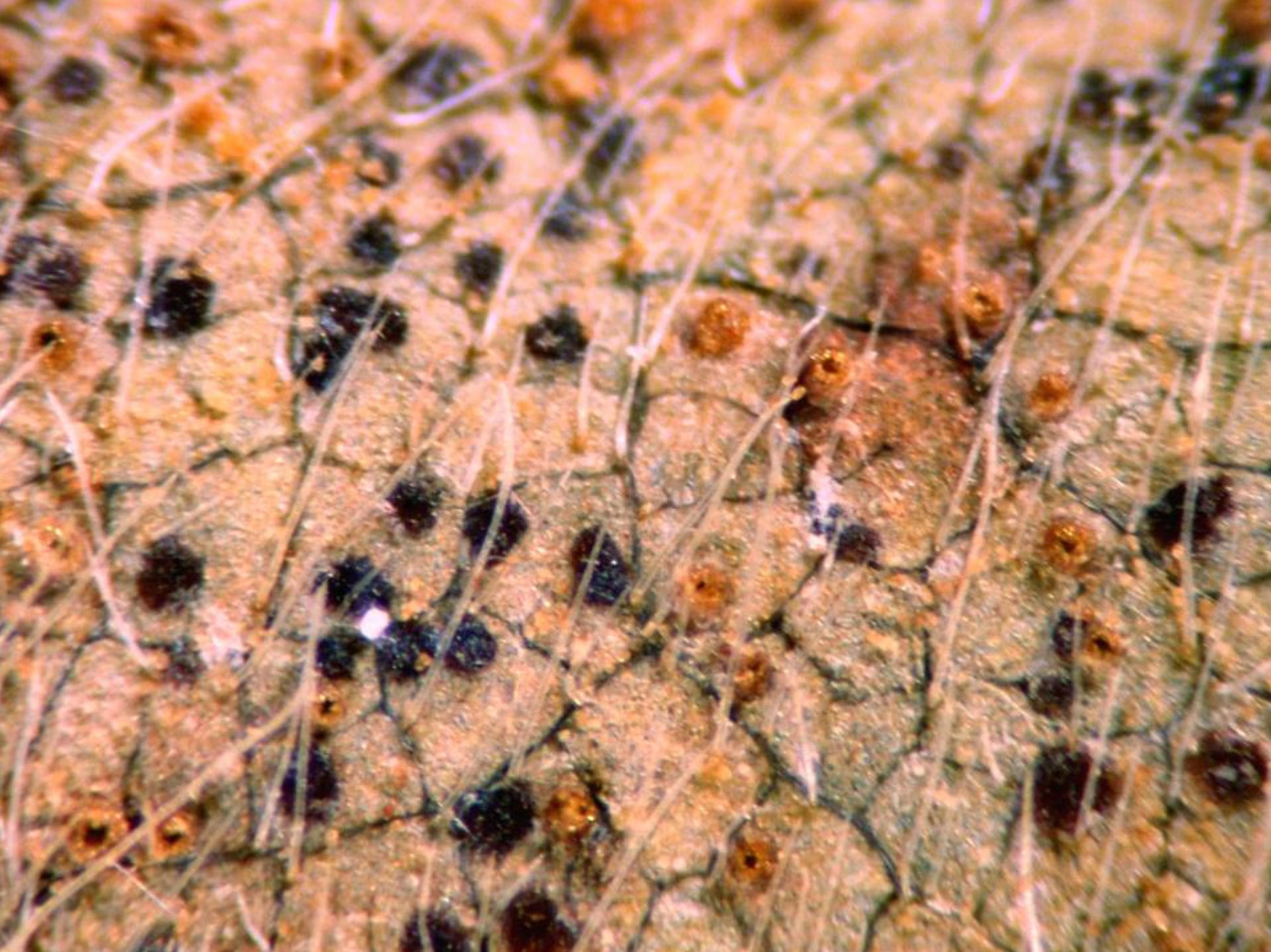
# To be continued...

Positive samples from three of six states when this was put together and only 5 in Mississippi

We are still collecting data and hope to publish the results when all data are collected.







# Thanks.

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# Questions?



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