

Analysis of rain samples for *Phakopsora pachyrhizi*

Les J. Szabo

Charlie Barnes

USDA ARS Cereal Disease Lab

Dept. of Plant Pathology

University of Minnesota

St. Paul, Minnesota

Van Bowersox

NADP

Illinois State Water Survey

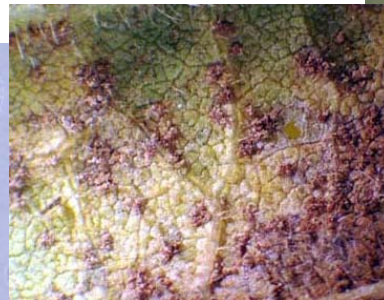
Champaign, IL

Jim Kurle

Dept. of Plant Pathology

University of Minnesota

St. Paul, Minnesota

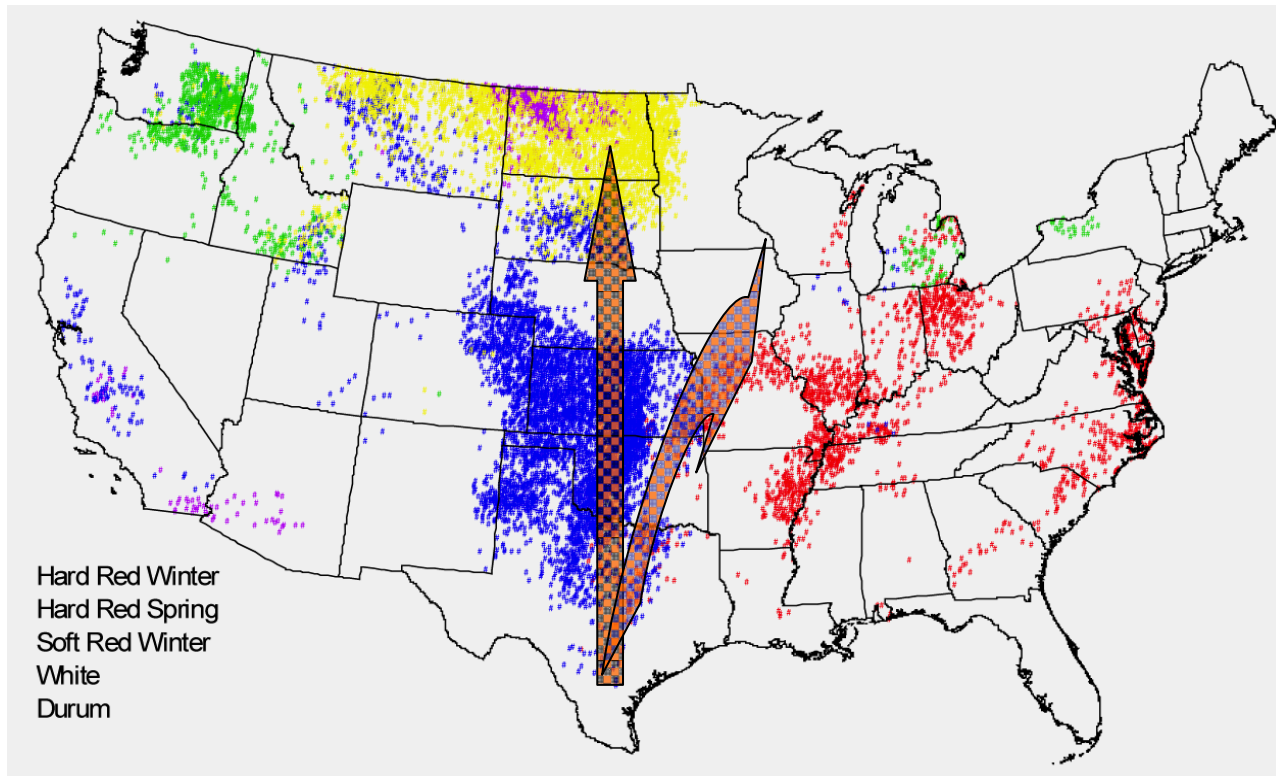


Introduction

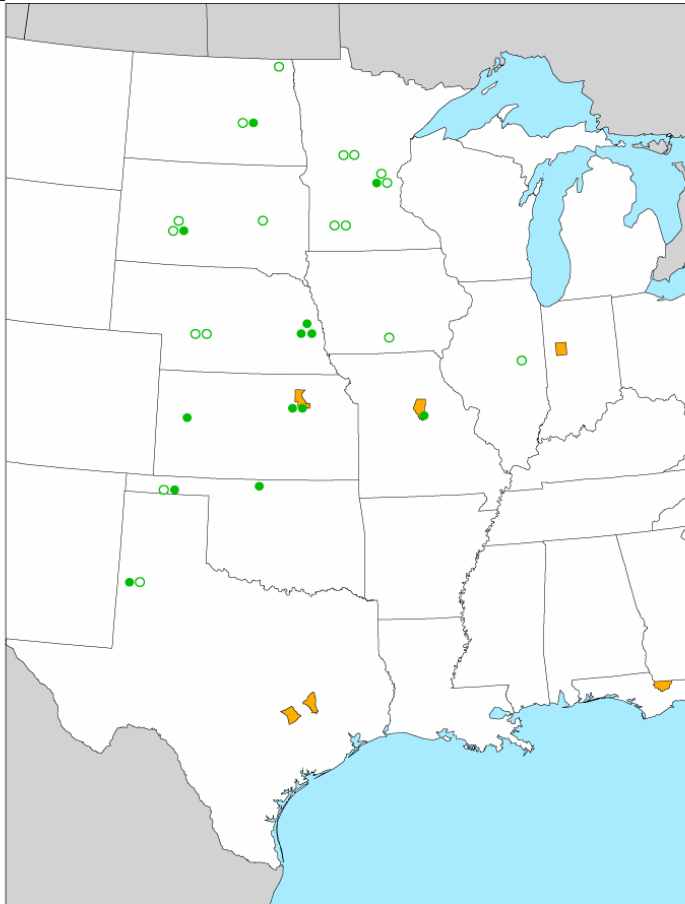
- Spores of rust fungi
 - Airborne
 - Travel long distances
 - *Puccinia graminis* (wheat stem rust)



Puccinia Pathway



Wheat stem rust



June 8, 2004



July 6, 2004

Rain Collectors

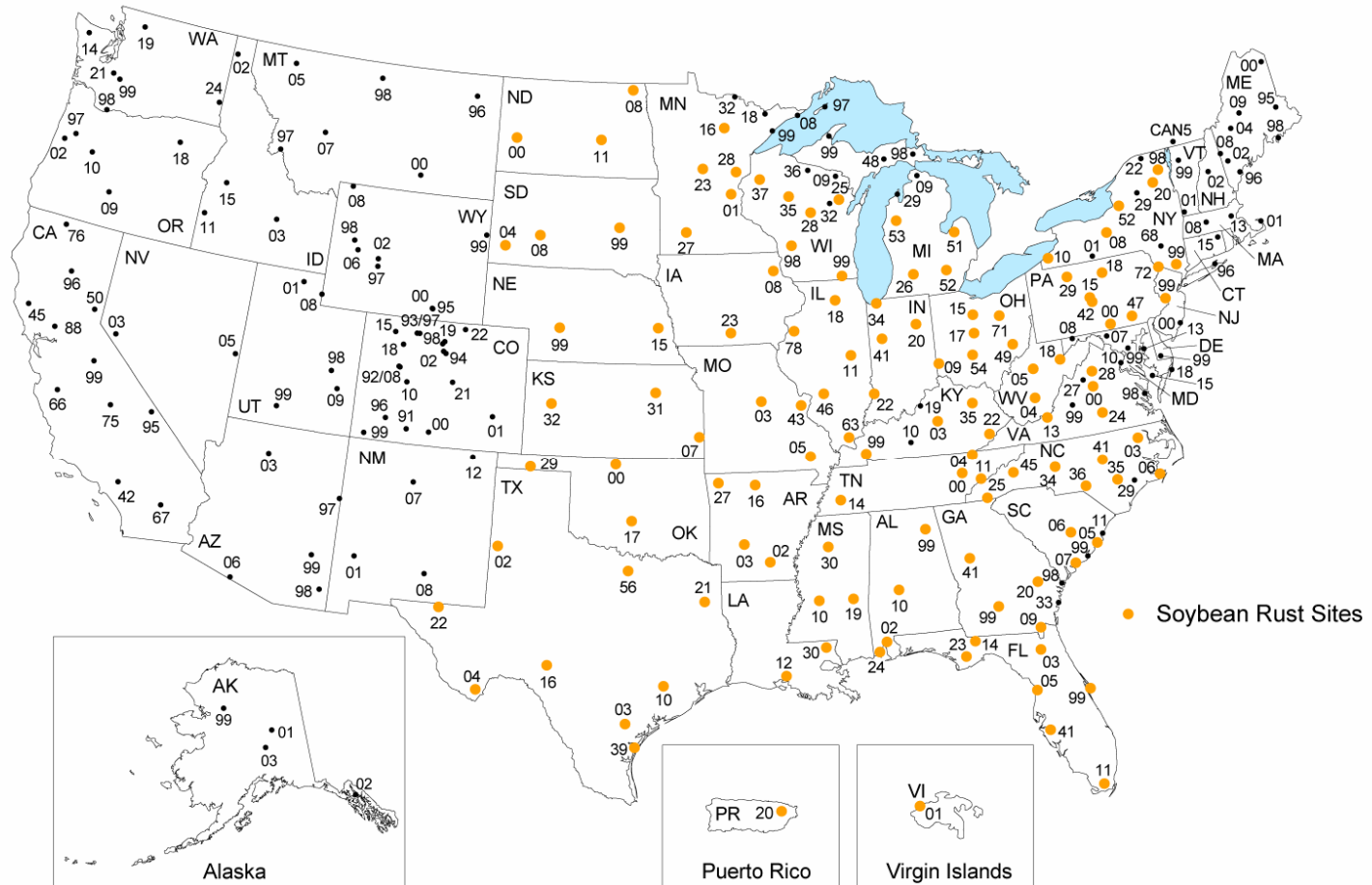


Rain collectors: NADP

- Active, collects only wet deposition
- Approximately 14" diameter
- 0.45 micron filter
- Weekly collections

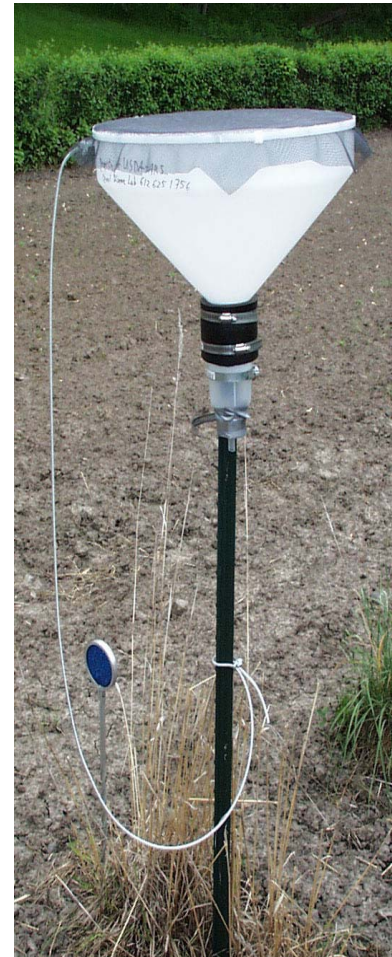


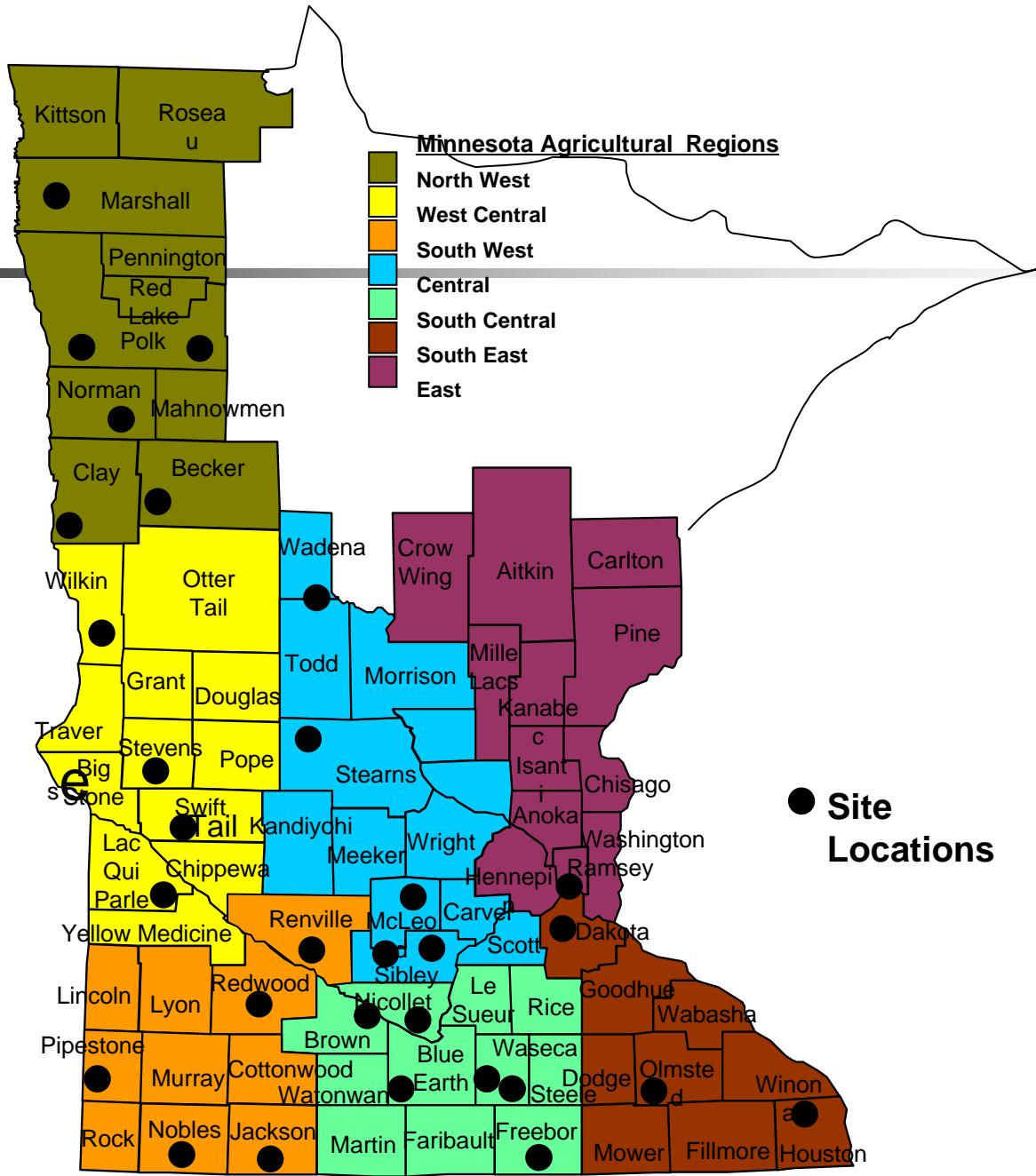
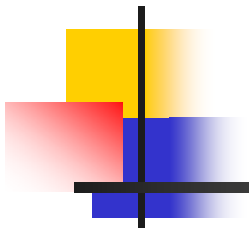
NADP/NTN sites



Rain collectors: JB

- Passive, collects both wet and dry deposition
- 17" diameter funnel
- Whatman filter holder
- 8 micron filter
- Filter holder changed weekly





Real-time PCR assay

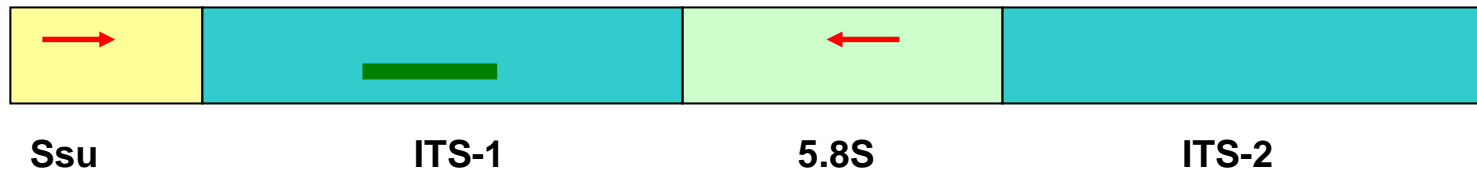


Real-time PCR assay

- Based on nuclear rDNA ITS region
- Single step assay
- Two step Nested assay

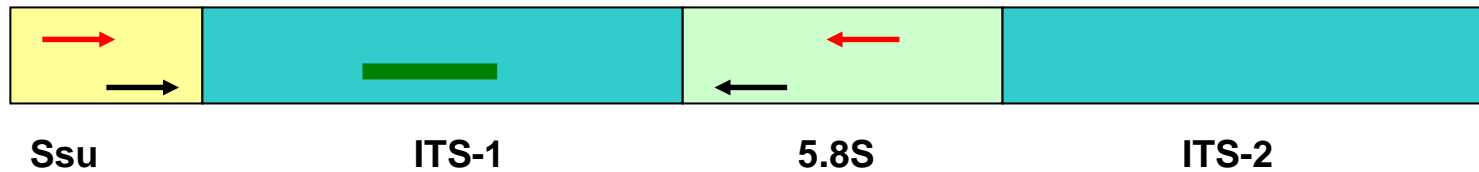


Real-time PCR assay



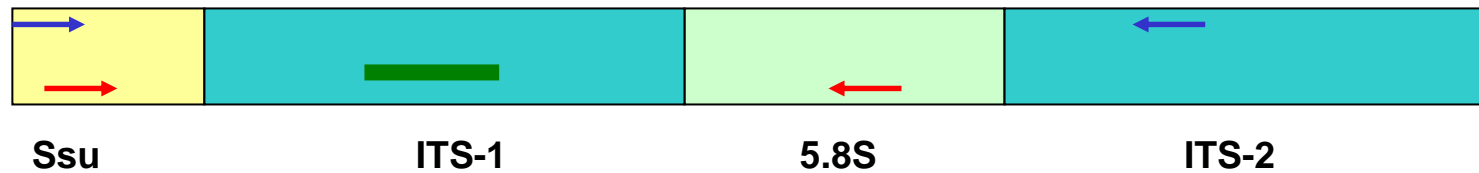
- One step
 - Rust specific primers
 - *Ph. pachyrhizi* specific TaqMan probe

Real-time PCR assay



- Nested-1 (Two step)
 - Rust specific primer pair (set 1)
 - *Ph. pachyrhizi* specific primer pair (set 2)
 - *Ph. pachyrhizi* specific TaqMan probe

Real-time PCR assay



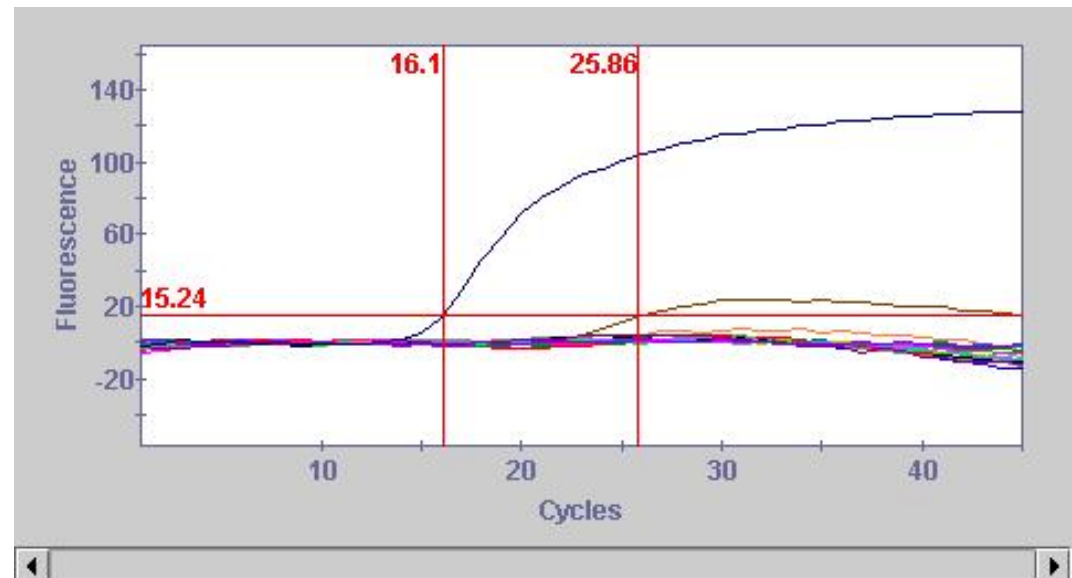
- Nested-2 (Two step)
 - *Ph. pachyrhizi* specific primer pair (set 1)
 - Rust specific primer pair (set 2)
 - *Ph. pachyrhizi* specific TaqMan probe

Real-time PCR assay

- Single step assay
 - *Ph. pachyrhizi* specific
 - Detection limit (approximately 200 fg DNA)
- Nested assay
 - *Ph. pachyrhizi* specific
 - Detection limit (< 1 fg DNA)
 - Nested-2 assay (*Ph. pachyrhizi* specific primer in PCR-1) was insensitive to high levels of other rust spores

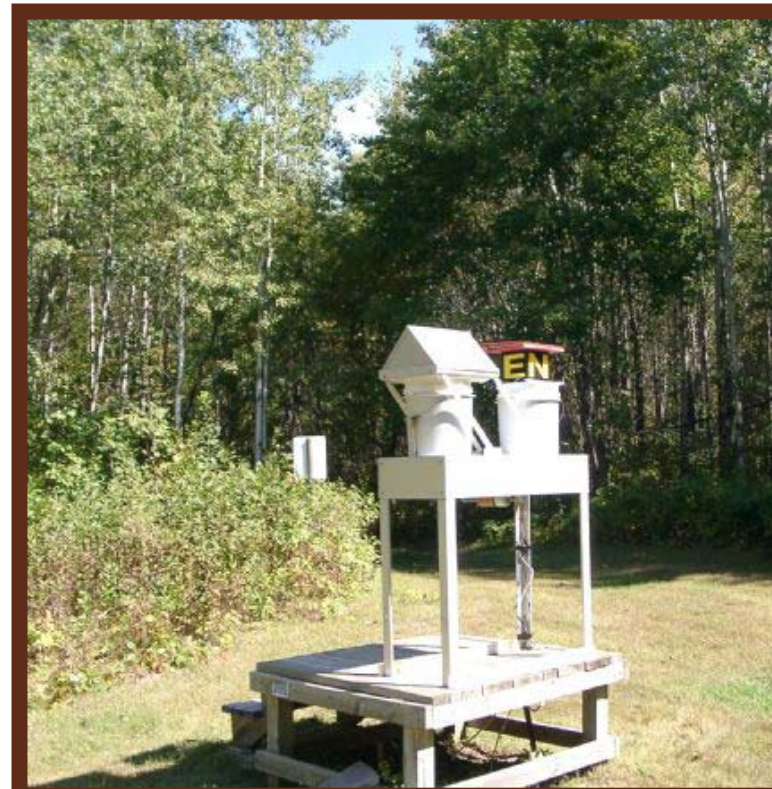


Real-time PCR assay: Results



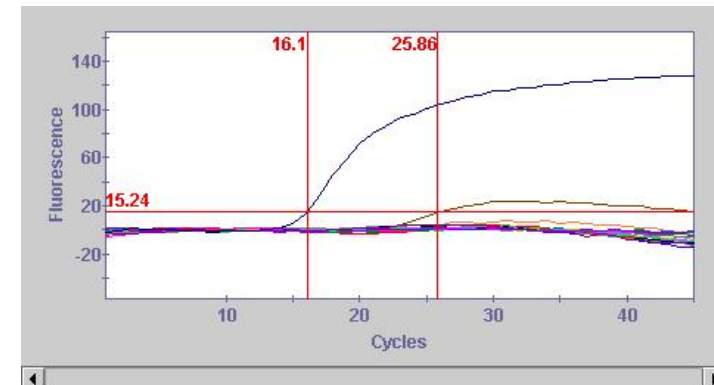
Real-time PCR: NADP

- 122 sites
- May 10 - October 8, 2005
- > 3,000 samples
- Processed >1,600 samples
 - May 10 - Aug. 30
 - 85 positive samples
- Amplification products of positive samples were analyzed on agarose gels
- Amplification products of selected positive samples were cloned and sequenced

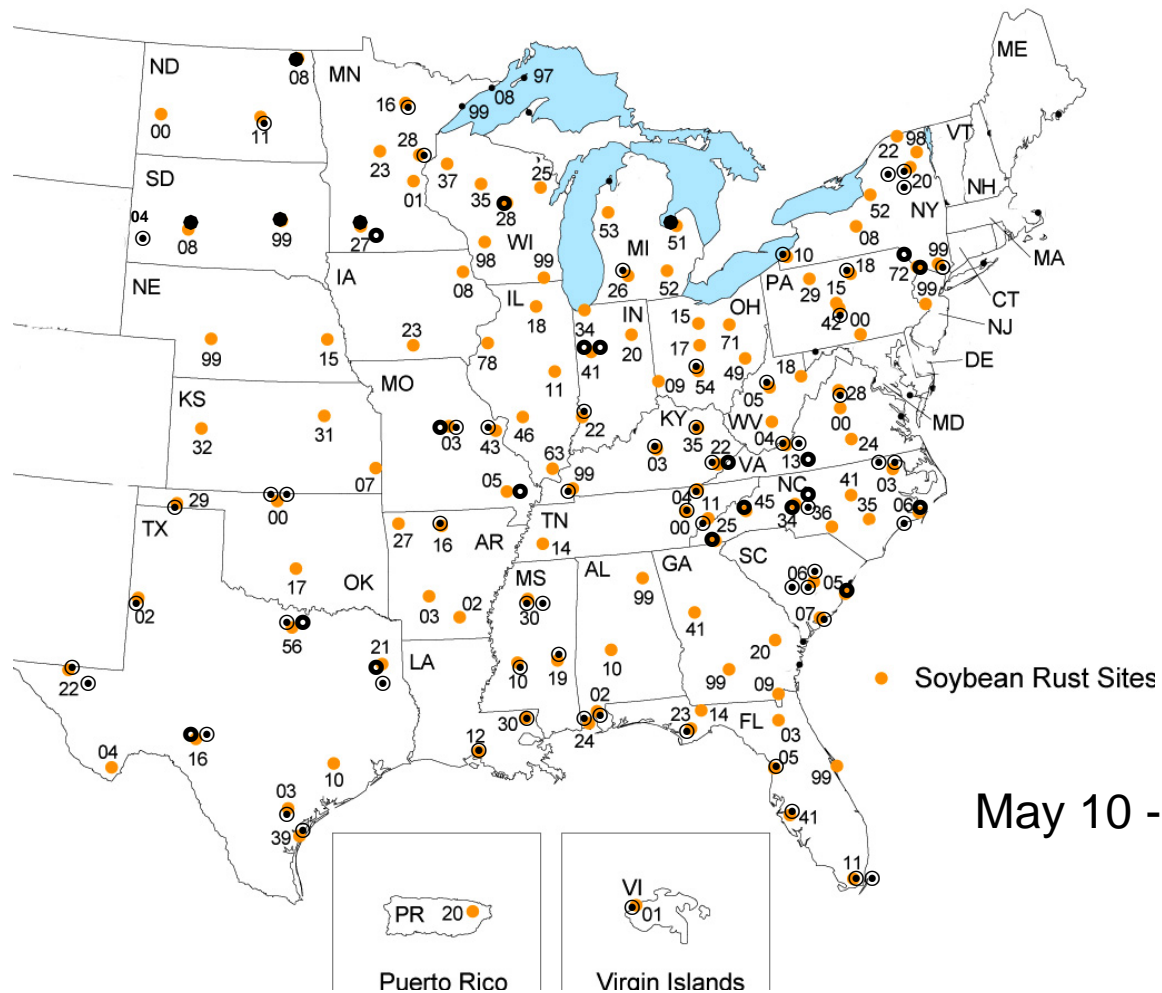


Real-time PCR:

- qPCR scale
 - 1 = trace (⊙)
 - FI > 10 SD above background
 - Usually no visible amplification product on gel
 - 2 = low (●)
 - FI > 15 SD above background
 - PCR amplification product visible on gel and correct size
 - 3 = moderate (●)
 - FI > 25 SD above background
 - PCR amplification product visible on gel and correct size



NADP sites: Positive samples



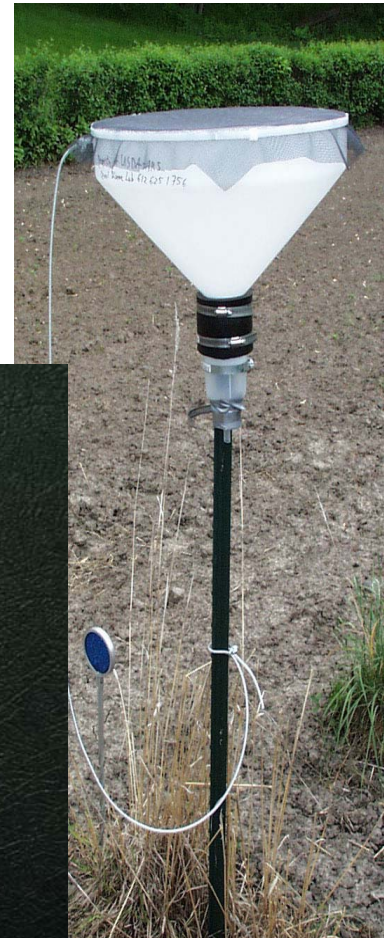
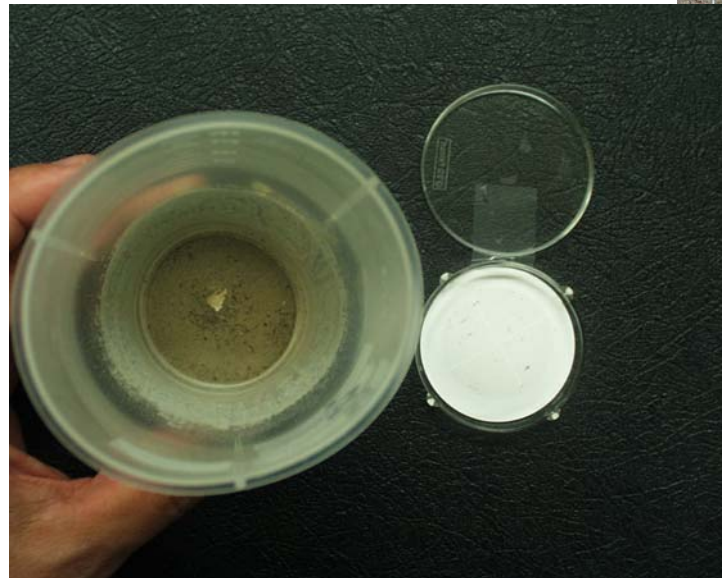
Real-time PCR assay: JB

- Samples from JB collectors are “dirtier” than samples from NADP collectors



Real-time PCR assay: JB

- Samples from JB collectors are “dirtier” than samples from NADP collectors
- DNA samples from JB samples show PCR inhibition using DNA spike experiments



Summary

- Real-time PCR assay was developed for testing rain samples for *Ph. pachyrhizi* DNA
- Rain samples from across the US soybean growing area were tested
- 85 samples tested positive for *Ph. pachyrhizi* DNA
- Positive samples were found in most of the regions test, including the Midwest and Northeast



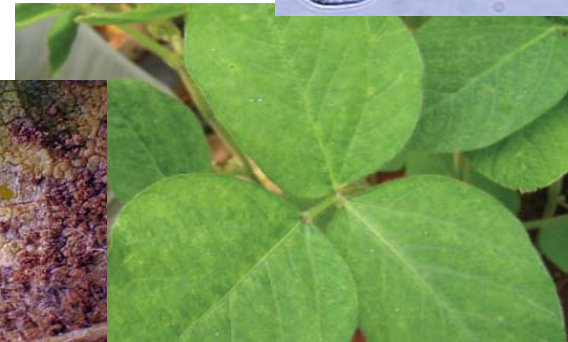
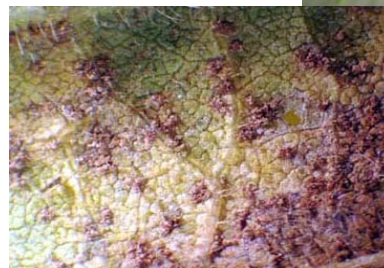
Summary (cont.)

- These results indicate that *Ph. pachyrhizi* spores are easily transported via atmosphere and can travel long distances
- Infection and disease development are complex processes and presence of spores (inoculum) is only one factor



Summary (cont.)

- Work that needs to be done
 - Calibrate Real-time PCR assay to actual amount of spores in sample
 - Finish processing remaining samples
 - Correlate PCR assay results with field data
 - Place NADP collectors at selected sentinel plots across the southeast
 - Refine protocol to accommodate “dirty” filters from JB collectors



Thanks

- USDA ARS CDL
 - Charlie Barnes
 - Jerry Johnson
 - Kim-Phuong Nguyen
- Dept. Plant Pathology, UMN
 - Jim Kurle
 - Crystal Floyd
 - Amy Holm
- NADP, ISWS
 - Van Bowersox
 - Karen Harlin
- Funding
 - USDA ARS
 - Minnesota Soybean Research & Promotion Council
 - United Soybean Board
 - Minnesota Agricultural Rapid Response Fund

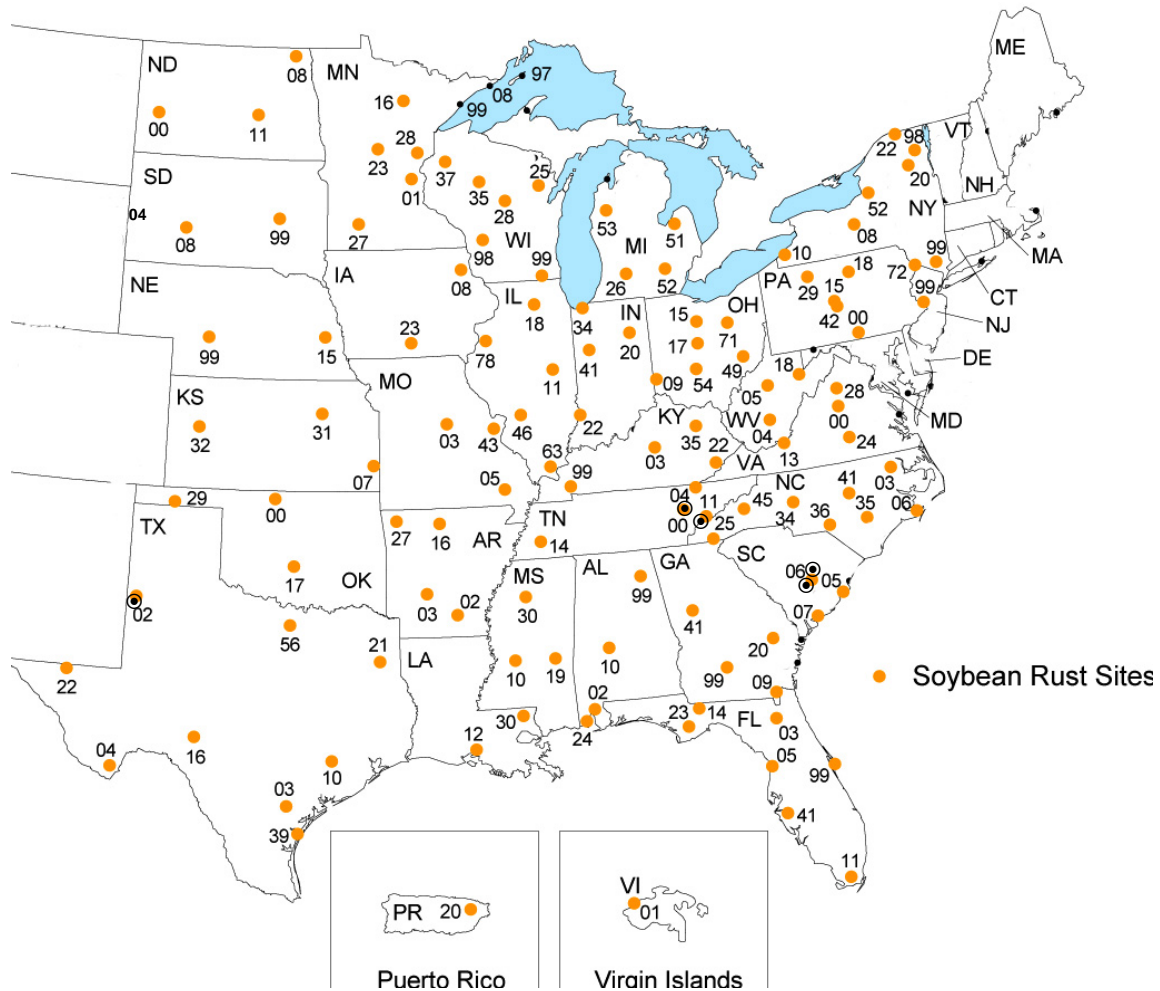


Processing filters

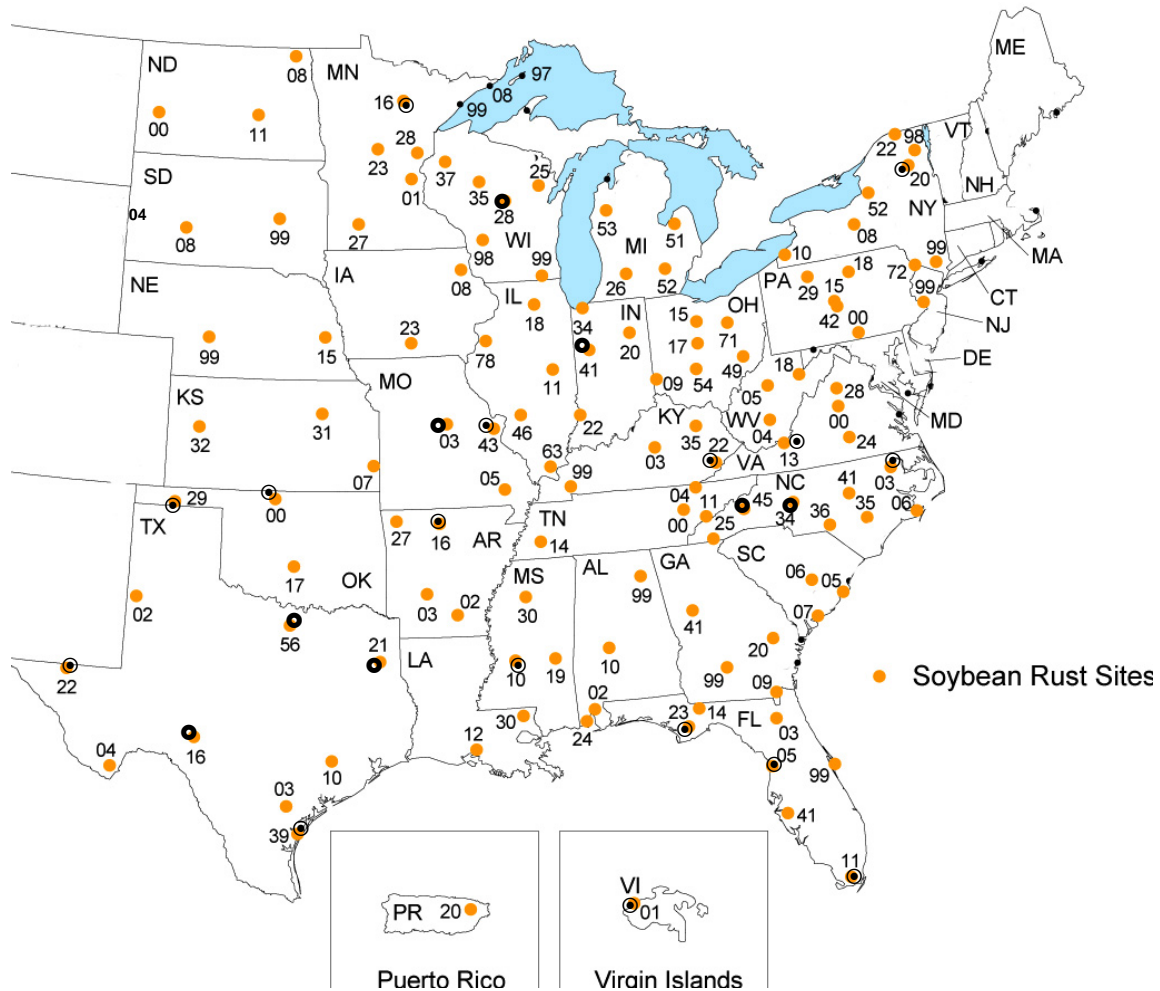
- Filters cut in half
 - 1/2 - DNA analysis
 - 1/2 - Archived
- Particulate matter removed by sonication
- Bead basher used to disrupt spores
- DNA extraction (Omni kit)



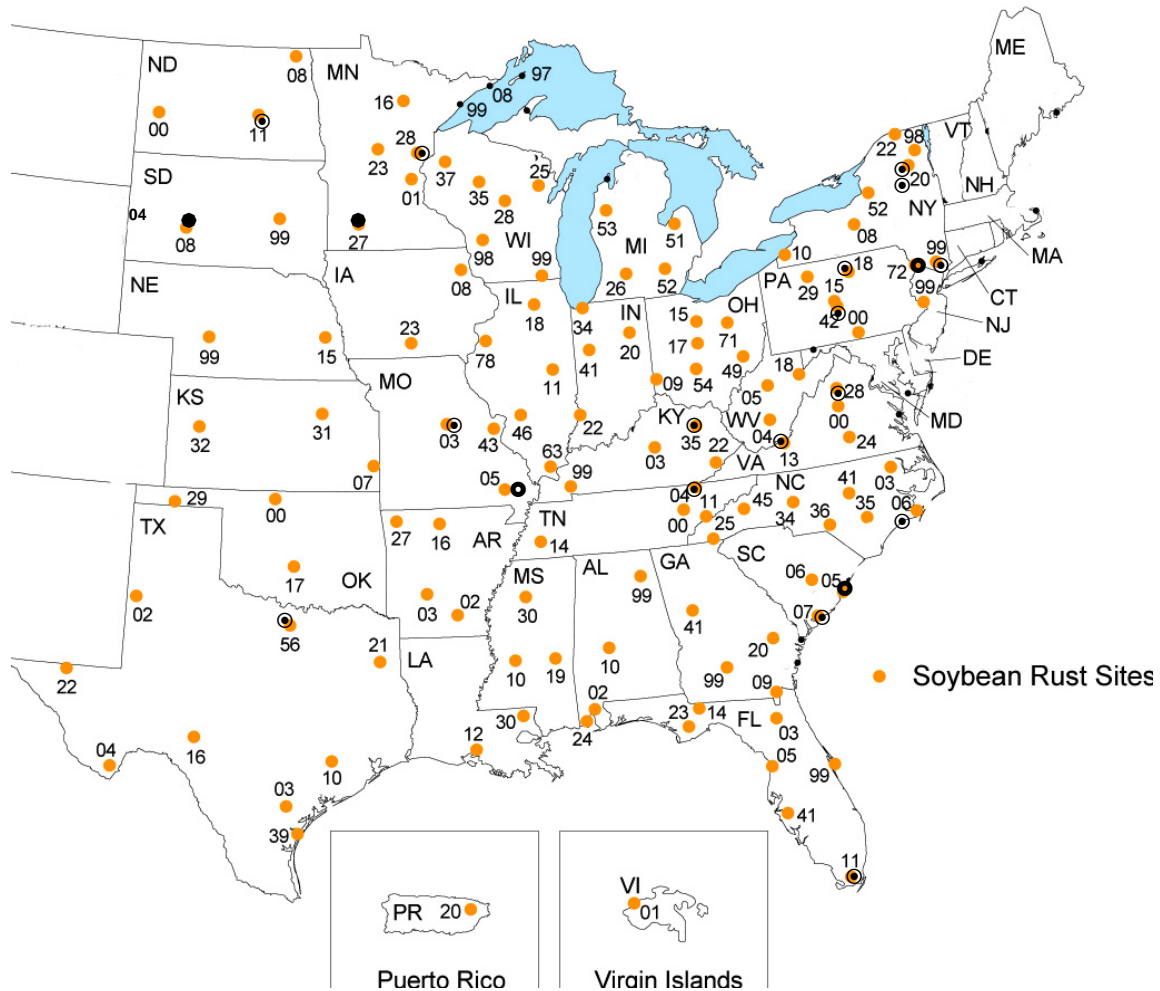
Positive samples: May



Positive samples: June



Positive samples: July



Positive samples: August

