New Approaches in Assessing Fusarium in California Cotton

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- Grower and PCA Cooperators
- National Cotton Council & Cotton Inc. State Support Committee, CA Department of Food and Agriculture
- seed companies

Fusarium in cotton at 7-9 node stage (Fungal disease caused by *Fusarium oxysporum f. sp. vasinfectum*)

Fusarium – *recognized and researched as a disease in cotton for years, so why look at it again?*

- **What is different, new, or newly recognized?**
- **First seen in finer-textured soils than typical for other FOV races assoc. with RKN – range of soil textures**
- **Confirmed that RKN pop'ns were very low (usually associated with damaging FOV popn's in CA cotton)**
- **Newly recognized strain for California (race 4)**
- **Few infected fields confirmed 2001-2004 (17 or 18)**
- **Mike Davis (UCD) and staff have been working on strain identification - has info on Australian strains as well as other races to identify that this is <u>not Australian FOV</u>**

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Fusarium - Questions / Concerns

- How identify this as a newly-recognized FOV race in California cotton? Why be interested?
- Could what we are seeing be related to damaging strains of FOV isolated in Australia? *At this point nothing identified has been of either of two known serious Australian FOV strains*
- What available methods can ascertain with more certainty different races of FOV dealing with?
  - *important in trying to assess potential for damage*
  - *Any value in identifying points of origin, sources?*
  - *Improved tools to look at genetics of host plant resistance*

1st Steps: Sampling fields, Collecting Information, When to Sample?

Symptoms can develop across a range of times and eventually lead to leaf abscisssion – foliar damage and leaf loss can occur over period as short as 1-2 weeks or much longer

Recognizing symptoms

Vascular staining *- easily seen in lower stem & upper tap root – how differ from verticillium vascular symptoms?*

1. *Seen more readily in root as well as lower stem*
2. *timing/growth stage when first seen much earlier*
3. *Staining tends to be more continuous rather than "flecking" or discontinuous discoloration*

Field Scale Symptoms:

Field 14-17a

- Typical field had affected areas about this size, with stunted "survivor" plants that produced harvestable bolls
- One 2003 field had much larger affected areas

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FIELD TRIALS: *Observations / activities summary 2002-2004*

- Evaluated sites where plants developed symptoms consistent with Fusarium *(about 65 fields in 2002, >80 in 2003, >40 in 2004).*
- *Samples collected in >25-30 fields each year – most confirmed as Fusarium races long-recognized in CA, but samples from about 12+ cotton fields in 2002-2004 were positive for race 4 FOV (about ¾ Pima, rest Acala in sandy loam to clay loam soil sites w/o Root Knot Nematode)*
- *Any fields where growers or PCA's contacted UCCE were evaluated for Fusarium, sampled if symptoms evident and permission given*

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Approaches in Identification

Field Sampling provided samples, so what is needed for ID and decision-making?

Identification / Genetic Fingerprinting:

- partial sequences of EF, B-tubilin, phosphate permase genes
- pathogenicity tests also conducted on plants grown in greenhouses
- isolates identified and stored, database maintained

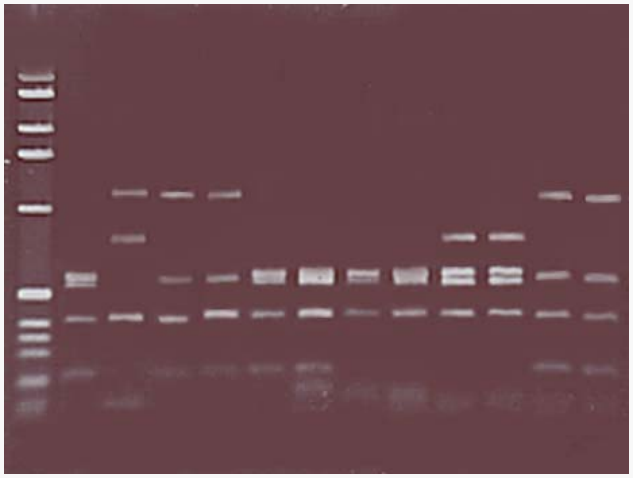
Approaches in Identification

Genetic Fingerprinting:

-IGS region of specific nuclear DNA amplified with specific primers

- amplified product digested by restriction enzymes *Scrf1* and *Rsa1*, and digested products separated by electrophoresis
- gels stained and examined to establish differences
- as develop information, may look in much more detail at base sequencing, markers and greater specificity re: sources of susceptibility and resistance

IGS/RsaI



Genotype

3
17 18
5 6 19 21
1 7 8 14 15

1 3 5 6 7 8 14 15 17 18 19 21
California Strains of FOV **Aust1** **Aust2**

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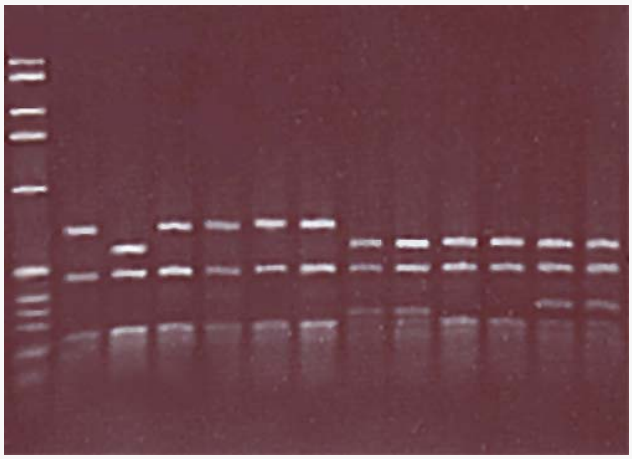
IGS/ScrfI



Genotype

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California Strains of FOV **Aust1** **Aust2**

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To be more certain with this analysis, can also look for more specific identification of where the genetics differ – by looking at DNA base sequence

Partial Sequence of EF gene

```
Aus 16: GACGGGG  CGCGTTTGCC  CTCCT.ACCA  TT...ACAAC  CT
Aus 17: GACGGGG  CGCGTTTGCC  CTCCT.ACCA  TT...ACAAC  CT
Aus 18: GACGGGG  CGCGTTTGCC  CTCCT.ACCA  TT...ACAAC  CT
Aus 19: GACGGGG  CGCGTTTGCC  CTCCT.ACCA  TT...ACAAC  CT
Aus 21: GACGGGG  CGCGTTTGCC  CTCCT.ACCA  TT...ACAAC  CT
Kgs  9: GAC.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCAGAAC  CT
Frs 14: GAC.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCAGAAC  CT
Tul  2: GAA.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCACAAC  CT
Krn  5: GAA.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCACAAC  CT
Kgs  8: GAA.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCACAAC  CT
Krn 10: GAA.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCACAAC  CT
Tul  1: GAC.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCACAAC  CT
Kgs  7: GAC.GGG  AGCGTTTGCC  CTCTT.ACCA  TTCTCACAAC  CT
Tul  3: GAC.GGG  AGCGTTTGCC  CTCTTAACCA  TTCTCACAAC  CT
```

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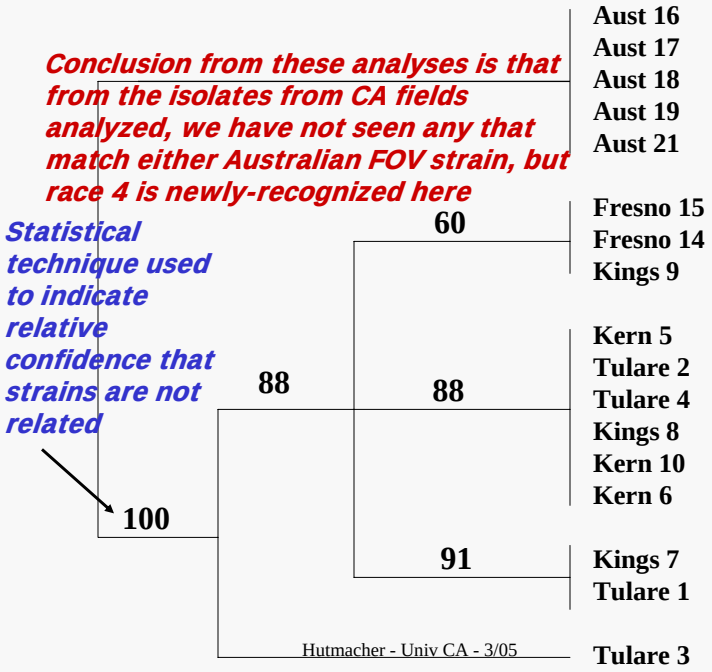
Conclusion from these analyses is that from the isolates from CA fields analyzed, we have not seen any that match either Australian FOV strain, but race 4 is newly-recognized here

Statistical technique used to indicate relative confidence that strains are not related



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California Cotton FOV's
(Identified to Date) **–** *Davis et al*

Races 1&2 (Americas)	Race 3 (Nile Valley)	Race 4 (Asia)	Race 8 (Asia)
Kern	Tulare	Kern	Kings
Kings		Fresno	Tulare
Tulare		Tulare	

No. of Race 4-infested Fields

	Fresno Co.	Tulare Co.	Kern Co.
2002-3	6	1	0
2004	6	1	4
Total	12	2	4

To deal with disease issues such as FOV race 4, efforts also needed in:

- Identifying if varietal resistance / tolerance is available or can be developed
- Containment practices / management to reduce rate of spread

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HOST PLANT RESISTANCE EVALUATIONS FOV RACE 4 – 2003 and 2004

- Fresno County (50 entries – 2003), (100 entries – 2004) 25' plots. Planted July 3 (2003), May 24 (2004)
- Kern County 24 entries, 3 reps with 15' plots. Planted May 26, 2004 (grower fields)
- Greenhouse 50 entries, total 9 plants/reps & 5 as control. During Oct. 19th to Dec 7th.

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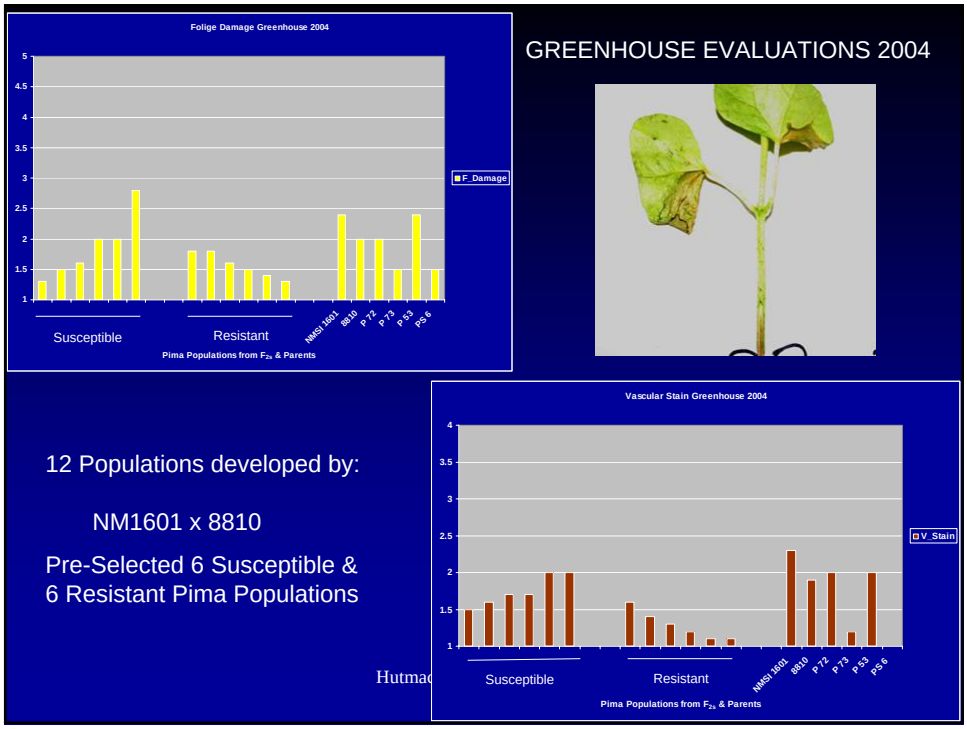
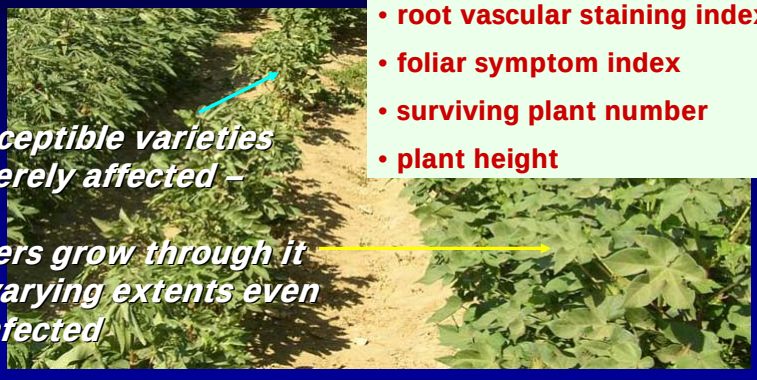


Field Variety Screens – FOV race 4 sites 2003-2004

Range of measurements made – focus here on:

- **root vascular staining index**
- **foliar symptom index**
- **surviving plant number**
- **plant height**



Susceptible varieties severely affected –

others grow through it to varying extents even if infected

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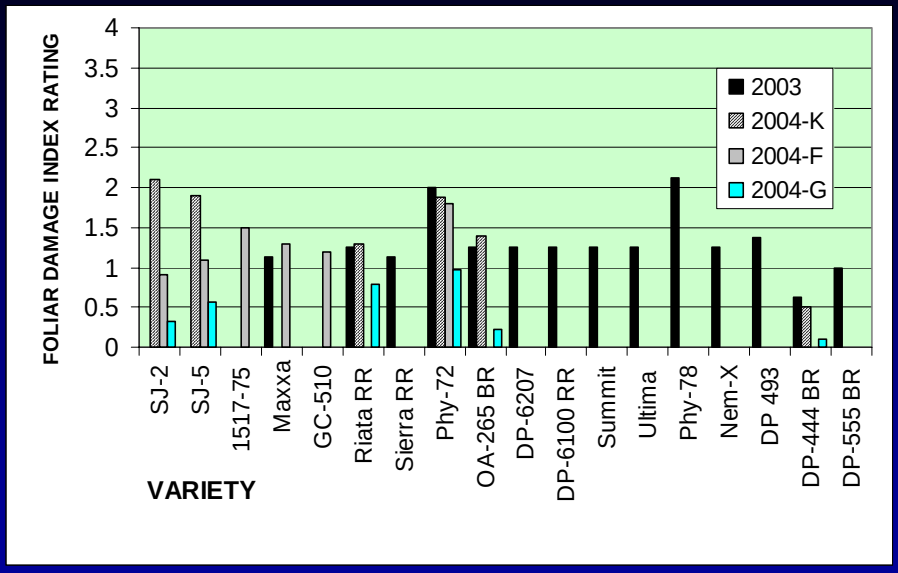
Foliar Damage Rating – Acala/Upland



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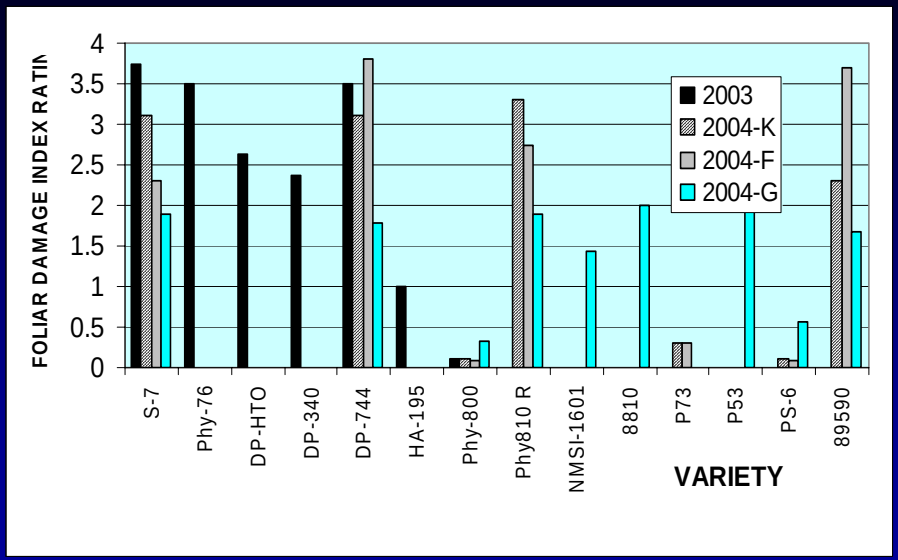
Foliar Damage Ratings – Pima entries



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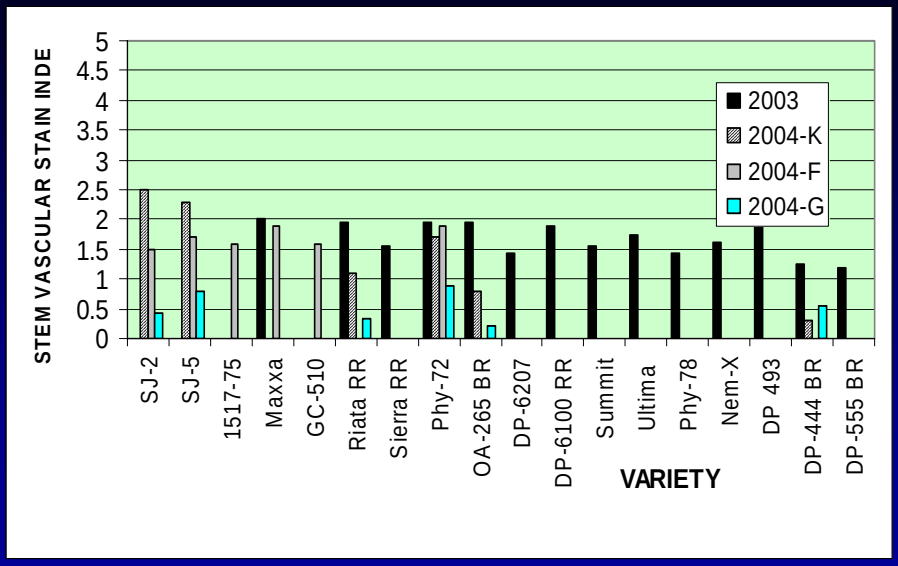
Stem Vascular Stain Rating–Acala/Upland



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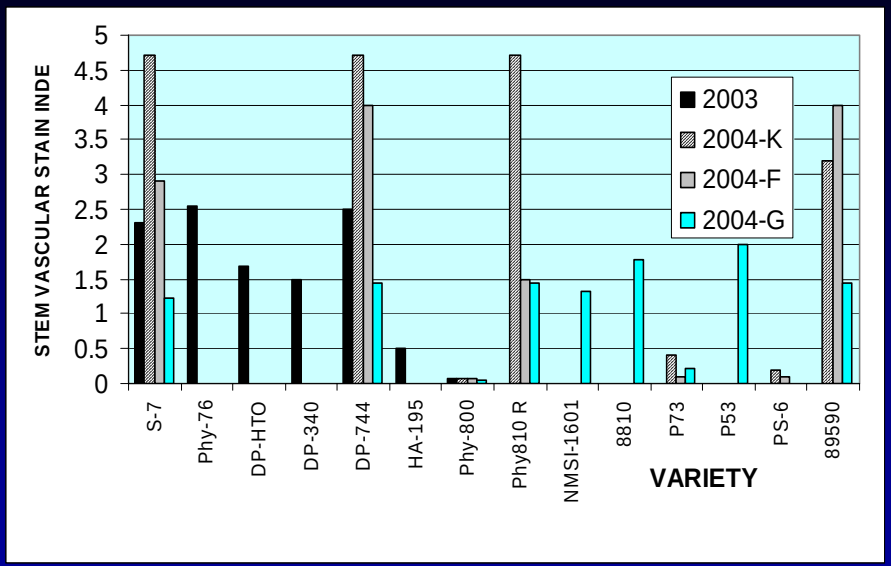
Stem Vascular Stain Rating – Pima entries



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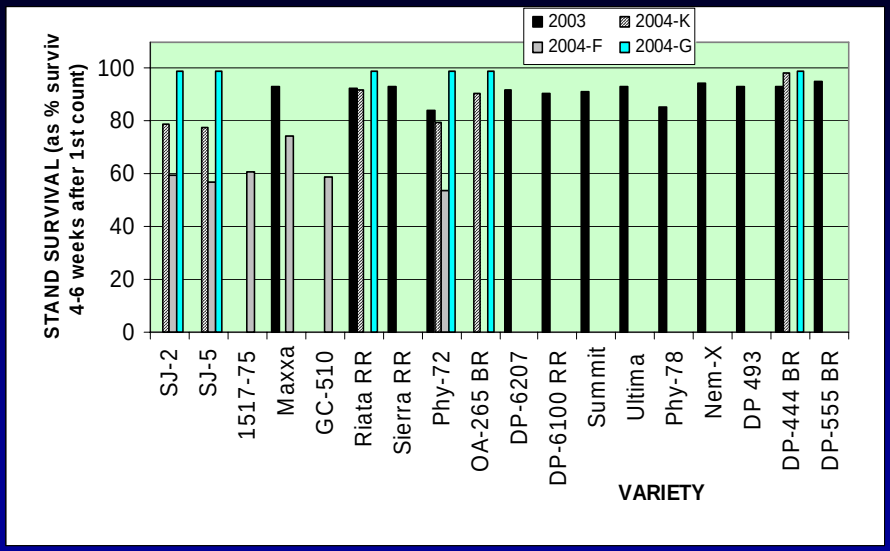
Plant Survival Percent – Acala / Upland



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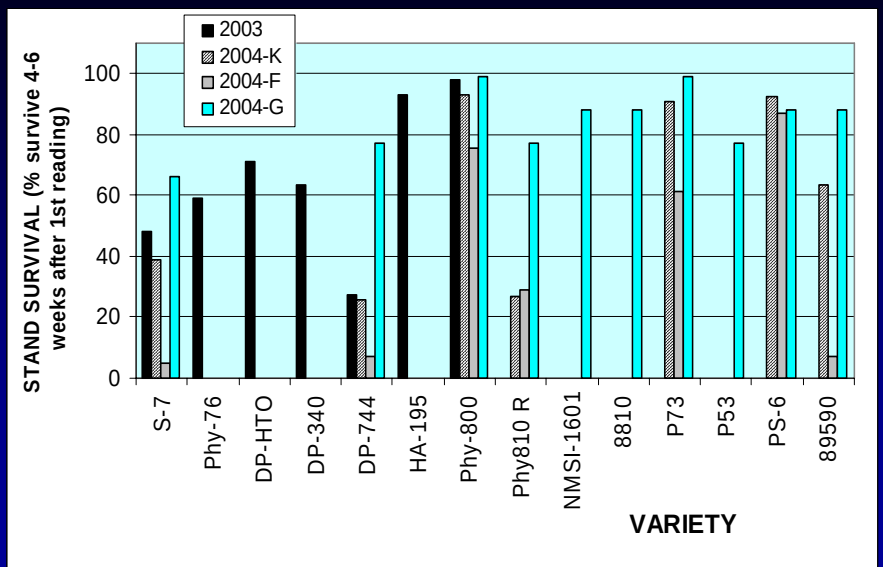
Plant Survival Percent – Pima Entries



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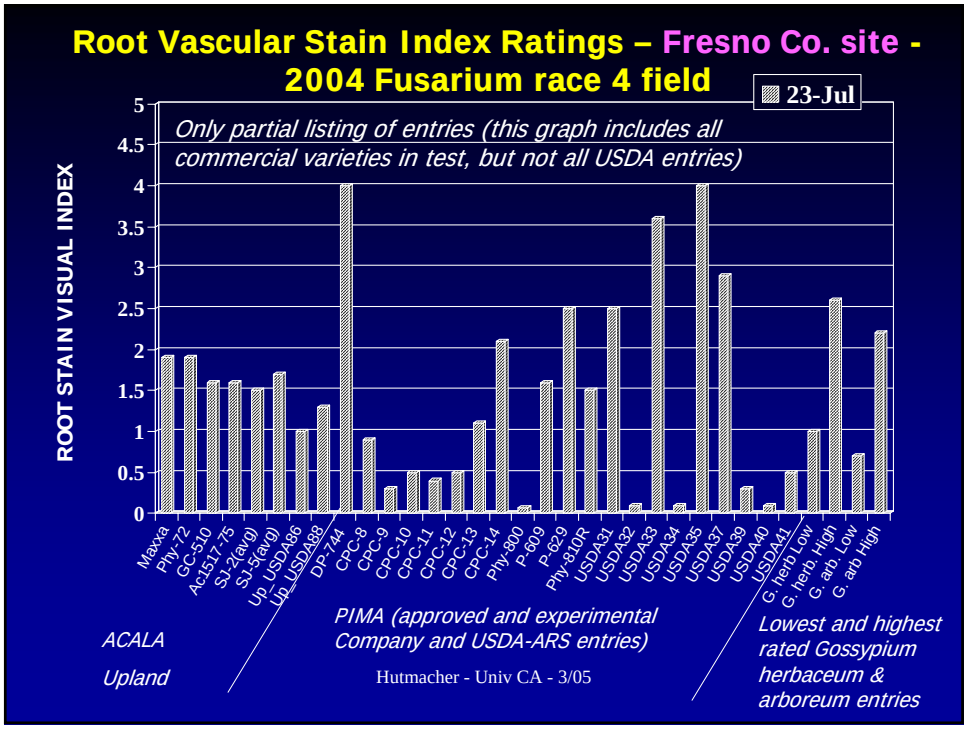
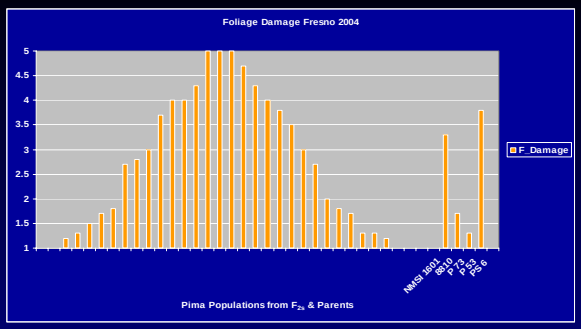
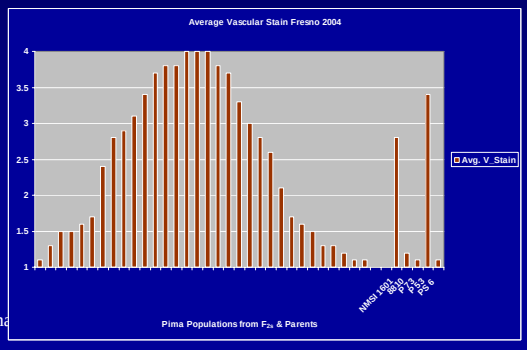




EXPAND INFORMATION DEVELOPED ON:

- **Heritability**
- **Potential Markers Useful in Selection and Improvement**



Summary – Identification Approaches

Genetic Fingerprinting:

- As develop more information, more detailed work on heritability questions, host plant resistance in public materials, identification of markers and greater specificity re: identification of sources of susceptibility and resistance

SUMMARY

- **FOV race new or newly recognized in CA cotton**
- **Many evaluated Pima varieties were observed to be more susceptible to FOV race 4 (stand loss, stunting, etc) than evaluated Gossypium hirsutums**
- **Preliminary results showed some Pima germplasm has a more complete resistance to FOV race 4.**
- **The impact of the disease for Acala / Upland cottons was milder than effects on most Pima, but still a problem, since those Acala / Uplands infected by FOV race 4 could reproduce & expand inoculum.**

SUMMARY

- **In Pima for this study, preliminary results showed that resistance for FOV race 4 may be inherited quantitatively and controlled by several genes.**
- **Heritability estimates indicated that selection for FOV race 4 can be accomplished.**
- **Early evaluations to look for highly resistant Acala and/or Upland cottons suggests that resistance in *G. hirsutum* may be more complex.**
- **Evaluations will continue**

Part of dealing with any disease still involves efforts to contain problems while more permanent solutions developed:

- **Identifying if varietal resistance / tolerance is available or can be developed**
- **Containment practices / management to reduce rate of spread**

Containment issues for growers as sites with FOV are identified ?

- *Remember that spores of this organism can be very long-lived so limit practices that expand movement*
- *What rotation crops will reduce inoculum / pop'ns most non-cotton crops will reduce inoculum levels, but unlikely any crop will eradicate it*
- *Can this strain influence other crop spp? Highly unlikely, but can impact Acalas/Uplands and Pimas*
- *Can inoculum be spread in fields with soil transport or movement of plant parts (leaves, flowers,squares?) ...yes.. By irrigation? ...yes... cultivation ...yes*

Containment / research issues

Improvements in detection methods *(could be useful in soil, and in plant tissues such as seeds – quick tests versus more in-depth methods)*

Develop and expand methods to provide general information (symptoms & recommendations, containment)

Example: UC cotton web site

http://cottoninfo.ucdavis.edu

Thank you