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 **not** Australian FOV

Hutmacher - Univ CA - 3/05

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

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

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:

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

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
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

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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
RSA Enzyme approach – (1) multiply DNA and “chop it up”; (2) use electrophoretic gel; (3) look at band separation to differentiate strains.

Differences in several locations – 2 primary Australian strains.

Focus on areas within DNA where opportunities to differentiate genotypes – data shows diversity in CA genotypes as well as differences from Australian strains.
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**

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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*
California Cotton FOV's

(Identified to Date) – Davis et al

<table>
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<tr>
<th>Races 1&amp;2 (Americas)</th>
<th>Race 3 (Nile Valley)</th>
<th>Race 4 (Asia)</th>
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No. of Race 4-infested Fields

<table>
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<th>Fresno Co.</th>
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<th>Kern Co.</th>
<th>Total</th>
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<tr>
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<td>12</td>
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</table>
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

HOST PLANT RESISTANCE EVALUATIONS
FOV RACE 4 – 2003 and 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.
12 Populations developed by:

NM1601 x 8810
Pre-Selected 6 Susceptible &
6 Resistant Pima Populations

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
Root Vascular Stain Index Ratings - Fresno Co. site - 2004 Fusarium race 4 field

Only partial listing of entries (this graph includes all commercial varieties in test, but not all USDA entries)

Foliage Damage Fresno 2004

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 reproduced & 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